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Number 19

# MARICULTURE RESEARCH UNDER THE CENTRE OF ADVANCED STUDIES IN MARICULTURE



CENTRE OF ADVANCED STUDIES IN MARICULTURE  
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE  
INDIAN COUNCIL OF AGRICULTURAL RESEARCH  
POST BOX No. 1912, COCHIN-682 018

The CENTRE OF ADVANCED STUDIES IN MARICULTURE was started in 1979 at the Central Marine Fisheries Research Institute, Cochin. This is one of the Sub-projects of the ICAR/UNDP project on 'Post-graduate Agricultural Education and Research'. The main objective of the CAS in Mariculture is to catalyse research and education in mariculture which forms a definite means and prospective sector to augment fish production of the country. The main functions of the Centre are to:

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- undertake collaboration programmes; and
- organise seminars and workshops.

Under the programmes of the Centre, Post-graduate courses leading to M.Sc. (Mariculture) and Ph.D. are offered in collaboration with the University of Cochin since 1980.

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## PREFACE

This Publication contains the research results, in brief, of the short-term research projects carried out by the students of M.Sc. (Mariculture) course of 1983 and 1984 academic sessions towards their dissertations in partial fulfilment of the degree.

The M.Sc. course in Mariculture is offered under the Centre of Advanced Studies in Mariculture at the Central Marine Fisheries Research Institute since 1980. This is a two-year programme and is conducted in close collaboration with the University of Cochin which awards the degree.

The structure of the M.Sc. course consists of a programme in basic sciences, marine biology, coastal hydrography, physiology, endocrinology and cytogenetics of marine animals; a general fisheries programme introducing the students to the foundation of marine, brackishwater and fresh water fisheries, fisheries economics and administration and fish and fishery biology; core programme on mariculture involving fish farm engineering technology and culture of finfishes, crustaceans, molluscs and seaweeds, management of mariculture and extension; and research methodology and preparation of dissertation on the basis of a short-term research project.

The main objective of the inclusion of a dissertation in the last semester of the course is to train the students in the research methodology and to develop their originality in planning of research, design of the experiments, collection of data and their interpretation. Besides these, the allocation of the research topics on the identified priority areas such as nutrition, physiology, pathology, genetics, reproductive physiology, and eco-physiology of cultivable marine organisms and on culture systems has also helped to either add to the basic knowledge in these aspects or to supplement the information required to tackle the applied aspects. It may also be noted that the emphasis in the present series of investigations has been on prawn culture, especially of the species *Penaeus indicus*. As a result of these programmes, we know, today more about this species and its culture aspects, than any other marine penaeid prawns of the country.

While the full scientific papers on the results of these investigations would be published elsewhere, the present publication attempts to summarise the salient features of observations and conclusions so that the information is made available at one place and the information could be utilised for the expansion of the knowledge on similar lines of investigations.

I would like to take this opportunity to record my great appreciation to all the students for their hard and sincere work put up and the originality shown by them in tackling the problems allocated to them. I also wish to thank my colleagues who have spared their valuable time in supervising and guiding the students' work.

**E. G. Silas**

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Cochin,  
December, 1984.

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**METABOLIC AND EXCRETORY RATES OF THE PRAWN  
*PENAEUS INDICUS* H. MILNE EDWARDS FED ON  
DIFFERENT TYPES OF NATURAL AND ARTIFICIAL  
FEEDS**

*Introduction*

A prerequisite for intensive aquaculture is the information on the basic physiology of the candidate species. One of the most important aspects in this regard is the integration of dietary parameters with that of the physiology of the candidate species. Also, fixing the percentage of protein in the diet is of great importance since it determines the growth of the animal and also the cost of the feed. Conventional growth studies although provide evidence for defining the specific nutritional requisites, often fall short in ascertaining an adequate description of nutrient processing in the tested organism. Failure to provide adequate explanations on physiological grounds hinders the complete understanding of the nutrition of the cultured species and the applicability of the tested diet in different environments. Determination of the optimal dietary formulations for efficient food utilization and growth thus demands a physiological approach to the nutritional problems. The most essential parameters in the nutritional physiology are the metabolic and excretory rates. These when related with the growth rate of an animal in response to a diet can provide the basis for development of a suitable methodology for standardisation and optimization of the nutritional components in the diet.

### Objectives

The present investigation was taken up with the following objectives.

1. To standardise the methodology involved in the study, *i.e.* for the estimation of metabolic and excretory rates in juveniles of *Penaeus indicus*.
2. To estimate the metabolic rate of *P. indicus* when fed diets with different protein levels.
3. To study the changes in ammonia excretion associated with dietary protein intake.
4. To study relative growth rates with different protein levels in the diet.
5. To integrate the observations on the above parameters and to understand interaction between protein levels in diet, specific dynamic action (SDA), excretory rate and growth rate in juveniles of the *P. indicus*.

### Material and methods

Juveniles of the prawn *Penaeus indicus* (30–60 mm length, 300–900 mg wet weight) were used in the present study. The prawns were acclimated to the experimental medium of salinity 20‰ for a minimum of seven days prior to the commencement of the experiment. The parameters measured were metabolic, excretory and growth rate in prawn fed with diets of varying protein content. One natural diet *i.e.* muscle of *P. indicus* and three pelleted feeds with protein contents approximately 20, 40 and 70% were tested. Each diet was tested in two groups of animals. The group I were fed *ad libitum* ( $\approx 11\%$  of the body weight) and group II, restricted rations ( $\approx 7\%$  of the body weight). Starved metabolic and ammonia excretion rates were first estimated on animals not fed for 24 hrs. This was followed by feeding and estimation of metabolic and excretory rates of the same fed animal. A minimum of three individuals in each aquaria per treatment were taken as replicates. For estimation of metabolic rate, a closed-type respiratory apparatus was designed and used. Excretion rates were measured in 550 ml of filtered water (20‰) aerated by ammonia free water. For growth studies each treatment had three replicates with 15 animals in each

aquaria and the duration of experiment was 21 days. Ammonia was estimated by phenol-hypochlorite spectrophotometric method of Solorzano (1969, *Limnol. Oceanogr.*, 14 (5): 799-801). Total nitrogen was determined by micro-kjeldal procedure and converted to protein.

Standardisation of the methodology was attempted to eliminate the high variability in metabolic and excretion rates. The parameters selected for the standardisation of metabolic rate were the positioning of the inlet and outlet in the respiratory chamber, the volume of respiratory chamber, number of animals per chamber, duration of measurement of oxygen consumption and the flushing rate. A more or less consistent and low coefficient of variation was taken as the criteria for selection of standard values. A respirometer was standardised keeping these in mind. The volume selected was 600 ml and it was sufficient in that measurable changes in oxygen consumption could be detected in 30 minutes, when a single individual was placed and at the same time the oxygen extraction coefficient did not rise above 0.1. In the estimation of excretory rates it was found that the rate between 0-4 showed a consistent pattern while that between 4-8 was erratic. Estimate of bacterial count and nitrate content revealed that these were due to differential build up of bacteria and conversion of ammonia to nitrate in the different aquaria. Therefore excretory rates were estimated for 0-4 hour only.

#### *Results and discussion*

The relationship between starved metabolic rate (ml/animal/hr) and body weight (mg) was found to be logarithmically linear ( $r=0.92$ ) and the value of 0.613 in the present study is comparable to the earlier reported values in *P. indicus* namely 0.604 of Subramanyam (1962, *Proc. Indian Acad. Sci.*, 55 (8): 152-161) and 0.501 of Kutty (1969, *FAO Fish. Rep.*, (57) 3: 957-969). The 'fed rates' were always higher than the 'starved rates' in both the groups tested with the four tested diets. The effect of tested diets on specific dynamic action (SDA) was tested by analysis of variance and was found to be significant ( $P<0.05$ ) in both groups. The SDA showed a positive linear relationship with the actual protein consumption per meal ( $r=0.94$  and  $0.89$  for group I and group II respectively). A multiple regression equation was computed to describe the relationship between SDA and starved metabolic rate, protein fed during experiment, prior feeding level and wet body weight ( $R^2=73.9\%$ ). Of

the above, the amount of protein fed during the experiment was found to be the most important factor influencing SDA (Standard partial correlation coefficient = 0.82).

The effect of diets on fed excretory rate was found to be significant when adjusted for the starved rates by analysis of covariance. A multiple regression equation was computed to describe the relationship between fed excretory rate and the starved rate, prior feeding level and actual protein consumed during the experiment ( $R^2 = 83.8\%$ ). The fed excretion rate was found to be primarily a function of starved excretory rate followed by protein consumed during the experiment.

The diets significantly affected growth in both groups ( $P < 0.01$ ). When fed *ad libitum*, the optimum protein level was 40% and under restricted rations there was a positive linear increase in growth with increasing protein level of the diet. The inter-relationship between growth rate and protein consumed, fed excretory rate, SDA and initial body weight was derived by multiple regression analysis and was found to be highly significant ( $R^2 = 84.3\%$ ). The initial weight had great influence on the growth rate, followed by the protein consumption, fed excretion rate of ammonia and SDA.

The increase in SDA and excretion rate with increase in protein level of diet have also been observed in other species (*Macrobrachium rosenbergii* by Nelson *et al.*, 1977, *Comp. Biochem. & physiol.*, 58 A: 319-327; *Homarus americanus* by Capuzzo and Lancaster, 1979, *Can. J. Zool.*, 57: 1845-1848). The optimum level of 40% arrived in the present study is comparable to that observed in other *Penaeus* species and to the level of 43 and 42.9 observed by Colvin (1976, *Aquaculture* 7: 315-326) and Ahmad Ali (1980, *Proc. Symp. Coastal Aquaculture*, 1: 321-328) respectively in the same species. The present study reveals that growth rate is negatively influenced by routine metabolism and SDA and positively by the percentage of protein in diet.

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## STUDIES ON THE EFFECT OF AMMONIA, NITRATE AND NITRITE ON THE LARVAE OF *PENAEUS INDICUS*

### *Objectives*

The present study was undertaken to understand the relative toxicity of ammonia, nitrite and nitrate to the various larval stages so that the water management practices in larval rearing could be rationalised. This study also aimed at establishing the safe levels of these nitrogen compounds in seawater used for successful rearing of the larvae in hatcheries.

### *Material and methods*

Two types of experiments were planned. (1) *Prolonged experiments* to study chronic toxicity of different levels of ammonia and nitrite in the medium to the larvae of *P. indicus* over a period of 8-9 days from the nauplius stage to the Mysis 3/postlarva I stage. The incipient  $LC_{50}$  i.e. the concentration at which 50% of the larvae died after 9 days was calculated. The  $EC_{50}$  value i.e. the concentration at which 50% of the larvae did not metamorphose into post-larvae was also estimated. In Experiment I larvae from a wild spawner were used and in Experiment II larvae from an eye-ablated spawner were used.

(2) *Short term exposure* to study acute toxicity. Each larval stage viz. the nauplius, protozoa and Mysis was exposed to different concentrations of ammonia, nitrite and nitrate. The minimum period of exposure was 24 hrs for all larval stages. But for protozoa the 48 hr  $LC_{50}$  and for the mysis stage the 72 hr  $LC_{50}$  were

also calculated. For this study larvae from a wild spawner were used. The spawners, in either case, were kept in polythene-lined tanks of 1 m diameter for spawning. Only healthy and active larvae were selected and used in the experiments.

#### *Lethal toxicity bioassays*

The test solutions were made up by dissolving requisite amounts of analar grade  $\text{NH}_4\text{Cl}$ ,  $\text{NaNO}_2$  and  $\text{NaNO}_3$  as sources of ammonia, nitrite and nitrate respectively. The experiments were conducted in 1 litre capacity beakers containing 800 ml of test solution. Each beaker contained 10 larvae and was aerated. The larvae were fed with a mixed culture of phytoplankton dominated by *Chaetoceros* sp. and *Thalassiosira* sp. Food is an important factor which determines the rate of growth and survival of the larvae and to ensure uniformity of feeding conditions in all the beakers the following procedure was adopted. Filtered seawater stored in a fibreglass tank was mixed with the phytoplankton culture in the ratio of 8:1 in a 50 litre capacity bin every day. The test solutions in the experimental beakers were renewed every day.

All the concentrations in both the experiments were triplicated. On the first day, observations on the condition of the larvae and mortality were made once in 12 hrs and thereafter once in 24 hrs.

The temperature, pH and salinity of the medium in the beakers were measured every day. Total ammonia was measured by the method of Solorzano (1969, *Limnol. and Oceanogr.*, 14 (5): 799-801) and the percentage of un-ionised ammonia was calculated according to Whitfield (1974, *J. Mar. Biol. Ass. U. K.*, 54: 565-580) taking into account the pH, temperature and salinity of the medium. Nitrite was measured by the method of Strickland and Parsons (1965, *Bull. Fish. Res. Bd. Can.*, 163: 1-311). The  $\text{LC}_{50}$  and  $\text{EC}_{10}$  values were calculated by weighted probit analysis (Finney, 1952, *Statistical method in biological assay*. Charles Griffin & Company Ltd., London, pp. 661).

#### *Results and conclusion*

The median lethal concentration ( $\text{LC}_{50}$ ) of ammonia, nitrite and nitrate with reference to the three larval stages are compared in Table 1.

The  $LC_{50}$  value calculated in the basis of the acute toxicity experiments throw light on the relative significance of the three inorganic nitrogen compounds in the rearing of the larvae of *Penaeus indicus*.

TABLE 1

Stage	Exposure time (hrs)	$LC_{50}$		
		Total ammonia (NH <sub>3</sub> -N) ppm	Nitrite ppm	Nitrate ppm
Nauplius	24	3.58 (0.29)	10.23	1170
Protozoa	24	17.86 (0.95)	20.43	<3165
	48	16.80 (1.18)	15.37	—
Mysis	24	46.01 (3.17)	33.87	—
	72	—	—	<3165

Nitrate is toxic to the larvae only in very high concentrations which are not ecologically significant to the penaeid larvae. The only situation where nitrate is deliberately added to the larval culture system is when NaNO<sub>3</sub> or KNO<sub>3</sub> are introduced to stimulate the growth of phytoplankton on which the larvae feed. But, the generally added amount never exceeds 3 ppm NO<sub>3</sub>-N, a level which is many orders of magnitude less than the level toxic to the nauplius stage. According to Wickins (1976, *Aquaculture*, 9: 19-37) even in recirculated water systems the nitrate levels do not exceed 94 ppm.

The tolerance of the larvae to ammonia and nitrite is the least in the nauplius stage. While ammonia is nearly three times more toxic than nitrite to the nauplius stage, nitrite seems to become more toxic in the late protozoa and the mysis stages. In the protozoa stage the decrease in 48 hr  $LC_{50}$  value compared to the 24 hr  $LC_{50}$  is more marked for nitrite than for ammonia indicating that the protozoa progressively become more sensitive to nitrite than to ammonia.

Further, it is apparent that the nauplius is the most sensitive stage (the weakest link in the series of larval stages) and although the protozoa and mysis are more resistant to these toxins any deterioration in water quality will affect the nauplius stage first and greatly influence the ultimate survival rate.

Following the recommendations of Sprague (1969, *Water Res.*, 3: 793-821) the incipient  $LC_{50}$  values of ammonia and nitrite toxicity for the larvae of *P. indicus* were calculated during the present study using the data obtained in the long term (9/10 days) chronic toxicity experiments. The estimated incipient  $LC_{50}$  values are given in Table 2.

It is immediately apparent that the incipient  $LC_{50}$  values were considerably lower than the 24 hr and 48 hr  $LC_{50}$  values estimated from the acute toxicity experiments.

TABLE 2

Experiment No.	Incipient $LC_{50}$	
	ammonia-N ( $NH_3$ -N) ppm	$NO_2$ -N ppm
I	1.45 (0.11)	0.78
II	11.99 (0.93)	3.29

It is also clear that nitrite is more toxic than ammonia to the larvae. The incipient  $LC_{50}$  values also reveal marked differences between the performance of the two broods. The larvae from the wild spawner which were used in Experiment I were very sensitive to nitrite and ammonia; their survival was poor, their rate of development was slower in as much as they did not metamorphose into postlarvae even after 10 days and many deformed nauplii were observed. The larvae from the eye-ablated spawner which were used in Experiment II were more tolerant of ammonia and nitrite; their survival was better, they transformed into postlarvae in 9 days and the incidence of abnormalities was very few.

The  $LC_{50}$  values including the incipient  $LC_{50}$  are based on mortality rates and measure quantal (all or none) responses; the sub-lethal effects of the toxins on the larvae are not revealed. A more sensitive index,  $EC_{50}$  or the *Median Effective Concentration* (i.e. the concentration at which 50% of the population showed any sub-lethal response, i.e. retardation of growth, etc.) has been used by some workers (Wickins, 1976, *Aquaculture*, 9: 19-37) to study sub-lethal effects. In the present study, the  $EC_{50}$  is taken as the concentration at which the rate of metamorphosis to postlarvae is reduced to 50% of the rate observed in the controls in 9 days. This index is related to the rate of larval development and is very relevant in the context of rearing of the larvae in hatcheries, where it is advantageous to have the larvae metamorphosing quickly and synchronously. The  $EC_{50}$  values calculated on the basis of Experiment II were 3.23 ppm ammonia-N (or 0.25 ppm  $NH_3$ -N at pH 8.1) and 1.8 ppm  $NO_2$ -N. These values are lower than the incipient  $LC_{50}$  calculated for the same experiment and gives a more sensitive estimate of the toxicity of ammonia and nitrite to the larvae.



Finally, some discussion of the results in relation to water quality requirements for hatchery rearing of penaeid larvae is warranted. Sprague (1971, *Water Res.*, 5: 245-266) while discussing the "safe levels" of toxicants for aquatic animals, pointed out that the incipient  $LC_{50}$  is a very important parameter in this connection. Safe levels have been calculated from incipient  $LC_{50}$  values by using an empirical 'application factor' which varies from 0.05-0.30. Sprague (1971, *Water Res.*, 5: 245-266) recommended 0.01 of incipient  $LC_{50}$  as the safe level of most of the toxicants. According to this recommendation the safe levels of ammonia and nitrite for rearing of the larvae of *Penaeus indicus* have been calculated as 1.12 ppm ammonia-N (0.093 ppm  $NH_3$ -N at pH 8.1) and 0.33 ppm  $NO_2$ -N (i.e. 0.1 of the estimated incipient  $LC_{50}$ ). Since  $EC_{50}$  was found to be a more sensitive index of toxicity which takes into consideration the sub-lethal effects as well, it is proposed that the safe levels could be taken as 0.1 of  $EC_{50}$ . Based on the  $EC_{50}$  values obtained during the present study the safe levels of ammonia and nitrite for rearing the larvae of *Penaeus indicus* are estimated as 0.32 ppm ammonia-N (0.025 ppm  $NH_3$ -N at pH 8.1) and 0.18 ppm  $NO_2$ -N. The ammonia and nitrite levels during the present study did not exceed 0.11 ppm ammonia-N (0.005 ppm  $NH_3$ -N) and 0.08 ppm  $NO_2$ -N, both well within the safe limits.

*Papers published based on this dissertation*

JAYASANKAR, P. AND M. S. MUTHU 1983. Toxicity of ammonia to the larvae of *Penaeus indicus* H. Milne Edwards. *Indian J. Fish.*, 30 (1): 1-12.

——— AND ——— 1983. Toxicity of nitrite to the larvae of *Penaeus indicus*. *Ibid.*, 30 (2) : 231-240.

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# 3

## OXYGEN CONSUMPTION AND AMMONIA EXCRETION OF *PENAEUS INDICUS* H. MILNE EDWARDS IN DIFFERENT SALINITIES

### *Introduction*

Adequate knowledge on the effects of environmental parameters on cultivable organisms is a necessary prerequisite for successfully culturing them on a large scale. There is also considerable information on the biology, production and culture of crustaceans, but aspects of ecophysiology of many of these animals have been not fully studied.

Oxygen consumption is a parameter often used as an index of metabolism and is therefore of basic importance in defining the energy budget of the animal. A measure of ammonia excretion is also important as a measure of protein degradation, protein being the costly component in the food of fishes and crustaceans. Besides, behavioural changes especially that indicated by random activity, are important in studying energy utilisation and survival.

### *Objectives*

The present work was taken up with a view to understand the metabolic, excretory and behavioural responses to different salinities in *Penaeus indicus* which is one of the most promising cultivable penaeid prawns available along both the coasts of India. Besides providing basic information on the influence of salinity on metabolism and activity, this study furnishes basic information of value in the aquaculture of this commercially important prawn.

### Material and methods

Adult male and non-ovigerous female intermoult specimens of *Penaeus indicus* ranging in length 130 mm–138 mm ( $133.74 \pm 2.56$  mm) and weighing 14.0 to 14.6 g ( $14.33 \pm 0.16$  g) were collected from the grow-out ponds of the Narakkal Prawn Culture Laboratory (NPCL) of the Central Marine Fisheries Research Institute, Cochin and used for this study. The salinity of the NPCL ponds at the time of collection of prawns was  $28 \pm 2$  ppt.

The prawns from the ponds were transferred to a pool containing sea water (34.0 ppt). The prawns from this pool were subsequently removed and were acclimated to different salinities namely 25.5 ppt, 17.0 ppt, 8.5 ppt, 4.2 ppt and 2.1 ppt. All the prawns were acclimated to a particular salinity for a period of 5 days before using them for the experiments and they were tested in the same salinity to which they were acclimated. The prawns in the acclimation tanks were fed with fresh clam meat *ad libitum*. They were starved for 24 hours before experiment. All the prawns were acclimated to and tested at  $28 \pm 1^\circ \text{C}$ .

Modified Fry's respirometer consisting of an electronic counter and a transparent plastic perspex respirometer was used for the present study.

The individual run of the experiment lasted for 60 minutes. The focus lights beamed at the photocells were switched on at least 60 minutes before starting day's experiment and remained on throughout the day of experiment. At the start of the experiment, initial samples were collected and the circulation of water through the respirometer was cut off. After an interval of 60 minutes final samples were collected. In each sampling time (initial and final of each run), two separate water samples were collected for analysis of dissolved oxygen and ammonia. The size of each sample was 50 ml for oxygen and ammonia (15 ml first collected as rinse was discarded). The figure in the activity counter was recorded immediately after sampling (initial and final sampling of all the runs). The random activity counts recorded by the electronic counter were also counter-checked by observing the movement of the prawn around the annulus of the respirometer.

After taking the final samples of the first run the respirometer was flushed with air-saturated water for a period of 15 minutes. Then the initial samples for the next run (as described above) were collected. In this manner the experiments were continued upto 12½ hours with the flushing intervals of 15 minutes in between.

All the experiments were conducted after acclimating the prawns to the respirometer overnight.

The concentration of oxygen and Ammonia were determined in the samples acquired at the beginning and at the end of each closure period. The activity was counted by the difference between the initial and final figure of the activity counter, which was noted immediately after each sampling. Modified Winkler's method was used for the estimation of oxygen and Ammonia was estimated by the phenol-hypochlorite spectrophotometric method.

#### *Results and discussion*

The mean rate of oxygen consumption in *P. indicus* in sea water (34 ppt) was 320.7 ml/Kg/hr, mean rate of ammonia excretion was 23.7 ml/Kg/hr mean Ammonia Quotient (A. Q. =  $\frac{\text{Volume of Ammonia excreted}}{\text{Volume of Oxygen consumed}}$ ) was 0.070 and mean random activity (counts/hour) as 10.5.

The mean rate of oxygen consumption in 25 ppt was 252.8 ml/Kg/hr, mean rate of ammonia excretion was 15.0 ml/Kg/hr, mean A.Q. was 0.060 and mean random activity was 11.5.

The mean rate of oxygen consumption in 17 ppt was 339.5 ml/Kg/hr, mean rate of ammonia excretion was 29.0 ml/Kg/hr, mean A.Q. was 0.085 and mean random activity was 14.8.

The mean rate of oxygen consumption in 8.5 ppt was 360.5 ml/Kg/hr, mean rate of ammonia excretion was 17.6 ml/Kg/hr, mean A.Q. was 0.015 and mean random activity 17.8.

The mean rate of oxygen consumption in 4.2 ppt was 384.3 ml/Kg/hr, mean rate of ammonia excretion was 41.6 ml/Kg/hr, mean A.Q. was 0.019 and mean random activity 19.9.

The mean rate of oxygen consumption in 2.1 ppt was 430.6 ml/Kg/hr, mean rate of ammonia excretion was 50.3 ml/Kg/hr, mean A.Q. was 0.117 and mean random activity 22.

Analysis of variance has shown that the difference in mean random activity at different levels of salinity is statistically significant. Similarly, the mean rates of A.Q. at different levels of salinity were also found to be statistically significant.

It is clear from the study that the rate of oxygen consumption increased with decrease in salinity of the medium except in 25.5 ppt. The rate of oxygen consumption was the highest in 2.1 ppt and the lowest in 25.5 ppt. The rate of ammonia excretion also increased with decrease in salinity of the medium except in 28.5 ppt. The rate of ammonia excretion was the highest in 2.1 ppt and the lowest in 25.5 ppt. The A.Q. values also increased with decrease in the salinity of the medium except in 25.5 ppt. The A.Q. value was highest in 2.1 ppt and the lowest in 25.5 ppt. The random activity also increased with decrease in the salinity of the medium. It was the highest in 2.1 ppt and the lowest in 34 ppt.

The results of the present study indicate that *P. indicus* spends least energy in 25.5 ppt and maximum in 2.1 ppt. The minimum rate of ammonia excretion in 25.5 ppt also indicates relatively lesser utilisation of proteins. The A.Q. values obtained indicate that relative protein degradation is minimum in 25.5 ppt and maximum in 2.1 ppt. The investigations also indicate that *P. indicus* expends more energy for activity in lower salinities.

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# 4

## MOBILISATION OF SOME OF THE METABOLITE RESERVES DURING MOULT CYCLE IN THE PRAWN *PENAEUS INDICUS* H. MILNE EDWARDS

### *Introduction*

Moulting, basically a means of growth for an animal with a rigid exoskeleton, is in fact a process which dominates a crustacean's life. The term 'moulting' used in a larger sense, includes all the processes of preparation for withdrawal from the old integument, ecdysis and post-ecdysial increase in linear size, as well as subsequent tissue growth. Moulting cannot be considered as a brief interruption of the normal life of the crustaceans, but as a process which has far reaching effects upon the whole physiology of the animal. In Crustacea, the periodic replacements of the integument and their underlying metabolite accumulation and tissue growth influence either directly or indirectly the metabolism, behaviour and reproduction of the animal.

### *Objectives*

In the present study, attempt has been made to understand better the moulting physiology of the Indian white prawn *Penaeus indicus* H. Milne Edwards, which is of primary importance for culture practices in the country. Since it is fundamental in any study of the moult cycle to know with a great precision each step of the moult cycle of the animal, an attempt has been made to classify the moult stages in *P. indicus* by a study of the setal development of the uropod. The various stages of the moult cycle are continuous, the recovery from one moult being initiation of storage of

metabolic reserves and preparation for the next moult. Thus, accumulation of reserves and their effective mobilisation in the course of the moult cycle has been recognised as one of the significant physiological characteristics of a typical decapod moult cycle. In the present study, quantitative variations in glycogen, protein, lipid and cholesterol contents of the muscle and hepatopancreas, variations in glucose, protein, lipid and cholesterol contents of the haemolymph and variations in the water content of the muscle in *P. indicus*, in response to the various stages during the moulting cycle have been measured. The organic reserves for the most part stored in the hepatopancreas are important not only as a source of material for the new exoskeleton, but also for the required energy during the moulting process. It is very evident that utilisation of these organic reserves ensure success of the moult as well as good growth at each moult in the crustaceans.

#### *Material and methods*

The prawn *P. indicus*, 80-100 mm in size, collected from the ponds at Prawn Culture Laboratory of CMFRI, Narakkal were used for the experimental purpose. Moult stage identifications were made by microscopic examination of the dorsal apical surface of the endopodite of the uropod and photo-micrographic pictures were taken. In the present study, the prawns for biochemical analyses were selected from 5 moult stages, viz. A. early post-moult; B. late post-moult; C. intermoult; D<sub>1</sub>. early premoult; D<sub>4</sub>. late premoult. Haemolymph collections were made from the pericardial cavity using hypodermic glass syringes and soon after, the prawn was dissected to excise out the hepatopancreas and muscle tissue. Biochemical estimations were carried out for quantitative determinations of lipid, cholesterol, protein and glycogen concentrations in the fresh hepatopancreas and muscle tissue and of lipid, cholesterol, protein and glucose concentrations in the haemolymph of animals belonging to the 5 different moult stages. The water content of the muscle tissue was also simultaneously determined.

The morphological changes associated with the setal development of the uropod in *P. indicus* were observed to be a good indicator for identification of different stages of the moult cycle. The presence of a translucent matrix and absence of internal cones in the setae were observed during the post-moult stages of A and B. In stage C, the prominent internal basal cone was the most significant feature. The onset of premoult, stage D was evident with

the separation of the epidermis from the cuticle at the bases of the setae indicating substage  $D_0$ . The stages  $D_1'$ ,  $D_1''$  and  $D_1'''$  were determined based on detailed studies on the morphology and extent of the newly forming setae. At the late premoult stage  $D_4$ , the new setae were fully formed and well developed and complete evagination of the new setae occurred during stage E, ecdysis, with the removal of the old exoskeleton in the process of moulting.

### *Results and discussion*

The lipid content of the muscle during the moult cycle ranged from 914.349 mg/100g to 1223.1135 mg/100g at stage  $D_4$  and stage  $D_1$  respectively. In the hepatopancreas, the lipid content was maximum during stage  $D_1$  and minimum during stage A, values being 2781.88 mg/100g and 1502.5135 mg/100g respectively. The lipid content in the haemolymph ranged from 143.533  $\mu$ g/100mg at stage A to 298.80  $\mu$ g/100 mg at stage  $D_1$ . In the muscle, the cholesterol content revealed a range of 1.034 mg/100mg to 1.774 mg/100g at stages A and  $D_1$  respectively. In the hepatopancreas, the cholesterol content was maximum at stage  $D_1$  (6.142 mg/100 mg) and minimum at stage A (3.7438 mg/100mg) respectively. The cholesterol content of the haemolymph showed a range of 4.80 mg/100ml to 12.2263 mg/100 ml, the maximum and minimum being at stages A and  $D_1$  respectively.

The protein content of the muscle during the moult cycle ranged from 35.881 mg/100mg to 43.389 mg/100mg. The protein content was lowest during stage A, gradually it increased to a maximum of 43.389 mg/100mg at stage  $D_1$  and then decreased again to 38.9525 mg/100mg at stage  $D_4$ . The protein content of the hepatopancreas showed a minimum of 28.4275 mg/100 mg at stage A and a maximum of 35.93 mg/100mg at stage  $D_1$ . In the haemolymph, during stage  $D_1$ , a maximum of 41.20 mg/100ml was observed and a minimum of 25.4583 mg/100 ml at stage A.

The glycogen content in muscle showed a maximum of 1.8541 mg/100 mg at stage  $D_1$  and with a minimum of 0.9787 mg/100 mg at stage A of the moult cycle. In the hepatopancreas, the glycogen content showed a range of 1.3575 mg/100 mg to 3.71951 mg/100 mg at stages A and  $D_1$  respectively.

In the haemolymph, glucose content was maximum during stage  $D_1$  and minimum at stage A, 94.84 mg/100 ml and 35.007 mg/100 ml respectively.



The water content of the muscle was the highest during stage A being 76.042% and the lowest of 71.218% at stage D<sub>1</sub> .

From the present study, it is now possible to stage the moult cycle of *P. indicus* using changes in the morphology of the setae during the moult cycle as a moult stage index. But further detailed studies are necessary to understand the genesis of the new developing setae which in turn demands more elaborate studies on cuticle histology and composition, structure and changing activities of the integument during the moult cycle of *P. indicus*. The aforementioned data indicate that there is a pronounced trend in the behaviour of the metabolic reserves with regard to the moulting cycle in the prawn *P. indicus*. The maximum values for all the organic reserves in the muscle, hepatopancreas and haemolymph were recorded at early premoult state D<sub>1</sub>, followed by a decrease at late premoult stage D<sub>4</sub>, indicating marked storage and subsequent utilisation during stages D<sub>1</sub> and D<sub>4</sub> respectively. The minimum values were recorded at stage A, early postmoult, indicating a depletion in the reserves just after moulting. The high percentage of water observed in the muscle during post-moult stage A and the low percentage recorded during stage D<sub>1</sub>, in contrast to the pattern observed for the organic reserves, clearly indicated the replacement of water by tissue growth during the periods between moults.

Consequent to the increasing demand of prawns, more attention has been paid for improving the technology of culture operations to produce more prawn in unit time and space. In this context, detailed studies on the moulting physiology of the candidate species will be of particular help. With more elaborate studies and a better understanding of the crustacean endocrinology coupled with the moulting physiology of any candidate species, acceleration of growth at different phases of culture may be achieved as the ultimate technology for obtaining higher production.

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# 5

## SALINITY TOLERANCE OF POSTLARVAE OF *PENAEUS INDICUS* H. MILNE EDWARDS

### *Introduction*

Salinity plays a vital role in the life processes of *Penaeus indicus* H. Milne Edwards. Although certain informations on the salinity requirements of the species are available, studies on larval and post-larval stages are inadequate.

### *Objectives*

The present study was undertaken to elucidate the optimum salinity ranges, relative survival rates at various levels of salinity, lethal salinity levels and effect of salinity acclimation on different size groups of the postlarvae.

### *Material and methods*

The study involved a series of rearing experiments in different salinity media with 3 different size groups of the postlarvae namely 8-9 mm, 13-15 mm and 18-19 mm total length raised at the Prawn Culture Laboratory of CMFRI, Narakkal. After acclimation in 20 ppt salinity for two days, the postlarvae were transferred into the test salinities at a stocking rate of 20 animals in 3 litres of the media in each container provided with constant aeration. The animals were fed with compounded dry pelletized diet developed at the Narakkal laboratory. All the salinity tolerance experiments were conducted in triplicate, each lasting for 120 hours (5 days) wherever survival extended throughout that period. The salinity acclimation experiments however lasted for longer periods. For every set of experiments a control series was also maintained at

20 ppt salinity in triplicate. The salinities in which at least 50% of the animals survived at the end of 120 hours exposure have been considered to be within the tolerance range and the rest as lethal.

Three types of experiments were carried out during this investigation. The first experiment was intended to find out the lowest as well as highest levels of salinity tolerated by the three postlarval groups and the survival rates in between on sudden transfer from a pre-acclimation salinity of 20 ppt to various test salinities between 0 and 60 ppt taken at 5 ppt intervals *i.e.* 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 ppt. In the light of the result obtained from these experiments the second type of experiment was conducted in salinity media between 1 and 5 ppt in the lower range and between 45 and 55 ppt in the upper range at 1 ppt intervals to locate the lethal salinities more precisely. The lower lethal salinity was studied separately for the 3 size groups while at the upper level it was attempted for the size group 13-15 mm alone. The data obtained were also statistically treated for estimating values of lethal salinities by following the 'Minimum Normit chi-square Procedure.' In the third experiment a series of rearing tests were conducted to determine the gain in salinity tolerance after the postlarvae were acclimated in various dilutions using the size group 13-15 mm. After acclimation in 20 ppt for 24 hours three batches of 20 animals each were released directly into the lethal salinity (2 ppt) and salinities below lethal (1 ppt and 0 ppt). After that, the stock of animals already acclimated in 20 ppt for 24 hours were brought down to the next acclimation salinity of 15 ppt from another with three batches of 20 postlarvae each were transferred to the test salinities (2, 1 and 0 ppt) after 24 hours and so on till down to 5 ppt acclimation and from 5 ppt the animals were acclimated to 3 ppt and then transferred to the test salinities in order to study the gain in salinity tolerance after gradual acclimation. At each salinity acclimation level the rearing lasted for 120 hours and the rate of survival was noted.

In addition to the above experiments, rearing was also conducted to find out the highest and the lowest salinity limits that can be tolerated by the postlarvae (19-20 mm) by stepwise acclimation to higher salinities upto 95 ppt and lower upto 0 ppt respectively from a pre-acclimation salinity of 20 ppt. The animals were maintained at each salinity level for 24 hours and after that the mortality was recorded.

All the above experiments were conducted at room temperature which ranged from 27.0 to 32.1°C. The water temperature of the experimental containers varied from 26°C to 30°C.

### Results and discussion

The survival rate of postlarvae in different test salinities at 5 ppt interval is depicted in Fig. 1. The size group 8-9 mm sur-

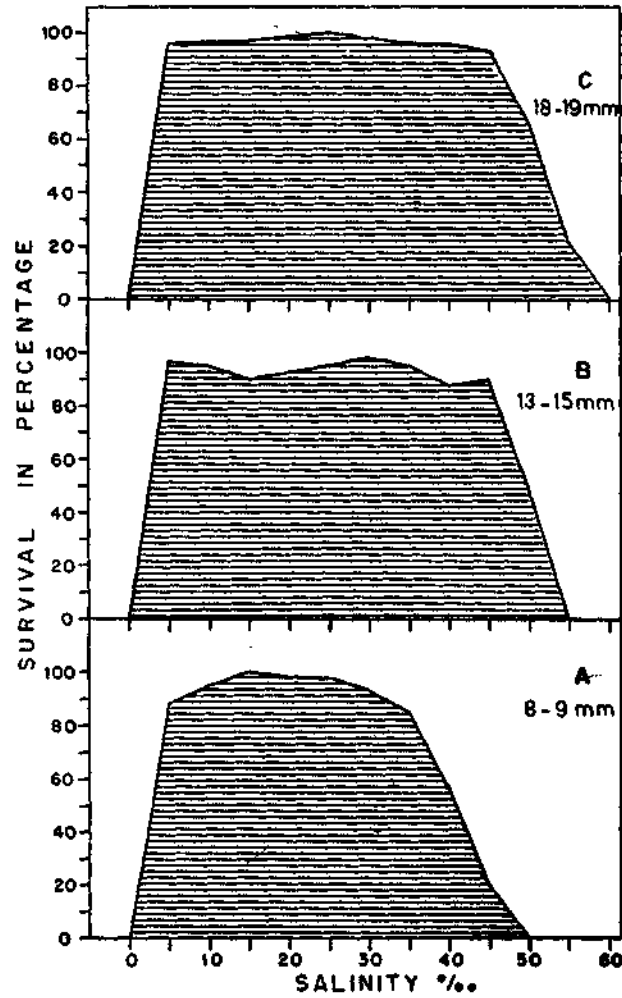


Fig. 1. Mean percentage survival of the postlarvae of *Penaeus indicus* in different test salinities between 0 ppt and 60 ppt at 5 ppt interval at the end of 120 hours.

vived well in salinities ranging from 5 ppt to 40 ppt, with maximum survival in 10-25 ppt. At 0 ppt all the postlarvae died within 30 to 45 minutes of their release. In 5 ppt salinity medium, the postlarvae showed gradual mortality and finally stabilized at 88% survival. Between salinities 10 ppt and 25 ppt the survival at the end of 120 hours was found to be above 95% with cent per cent survival at 15 ppt throughout the experiment. In the higher salinity levels, the decline in percentage survival was noticed from 30 ppt onwards. At the end of the experiment the survival in the medium of 30 ppt was 93%. In 35 ppt, 88% of the postlarvae were found to survive at the end of 24 hours of exposure and thereafter showed a gradual reduction in survival in the subsequent periods. At the end of 120 hours 85% of postlarvae survived. The mortality rate beyond this level of salinity was considerably high right from the first day of release, the final survival at 40 ppt being 56.7%. In the experiment in 45 ppt medium the survival rate of the postlarvae fell to 37% at the end of 12 hours and further reduced to 23% at the end of 48 hours. At 50 ppt, 75% of the animals died within 3 hours and only 2% survived by the end of 12 hours of the experiment and all the postlarvae died at the end of 36 hours of exposure in this medium. In still higher levels of 55 and 60 ppt salinity the postlarvae survived only for short duration not exceeding 45 minutes.

The post larvae of 13-15 mm size were found to tolerate a salinity range from 5 to 45 ppt. In most of the test salinities in this range the percentage survival at the end of 120 hours of experiment was above 90%. The highest percentage survival was registered at 30 ppt. Greater mortality rate was noticed from 50 ppt onwards when more than 30% of the postlarvae died within 24 hours of exposure. However, at the end of the experiment a survival of 48% of postlarvae of this size group was observed in this salinity although the postlarvae of the previous size group could not tolerate this high salinity. In 55 and 60 ppt salinity media the postlarvae did not survive more than 45 minutes as noticed in 0 ppt salinity.

The favourable salinity range for larger postlarvae belonging to 18-19 mm size was found between 5 ppt and 50 ppt. The percentage survival in different salinity media between 5 ppt and 45 ppt at the end of the experiment ranged from 93 to 100. At the 50 ppt salinity the survival fell to 75% at the end of 12 hours and to 67% at the end of 72 hours. All the surviving postlarvae could withstand this salinity level throughout the period of experiment. A sudden drop in the rate of survival with further increase in salinity was first observed at the end of 24 hours of experiment in 55

ppt salinity. At the end of 120 hours of exposure only 22% survived at this salinity. The post larvae hardly survived for 60 to 70 minutes at 0 ppt and 60 ppt.

It can be seen from Figs. 2 and 3 that the three size groups studied exhibited marked variation with regard to the rate of survival in progressive dilutions below 5 ppt salinity. In the case of 8-9 mm size postlarvae 70% survived till the end of 120 hours in

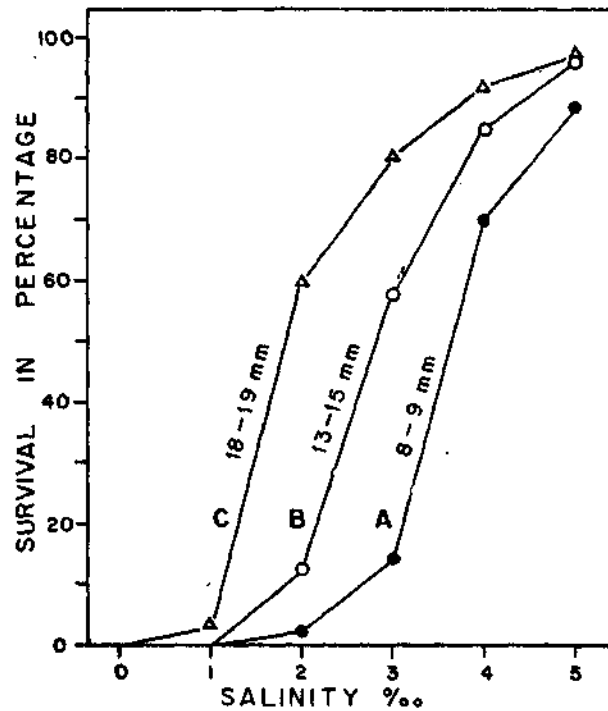


Fig. 2. Mean percentage survival of the postlarvae of *Penaeus indicus* in different test salinities between 0 ppt and 5 ppt at 1 ppt interval at the end of 120 hours.

4 ppt, while at 3 ppt salinity the survival fell down to 47% at the end of 12 hours and to 25% at the end of 120 hours. Since the percentage survival at the end of the experiment was less than 50% in 3 ppt this level of salinity can be considered as lethal for this size group. In the case of postlarvae of size 13-15 mm, the minimum salinity tolerated was found to be 3 ppt in which 63% of the post-

larvae survived till the end of the experiment, whereas in 2 ppt salinity only 25% survived at the end of 120 hours of the exposure. This evidently showed that 2 ppt salinity was lethal to this size group

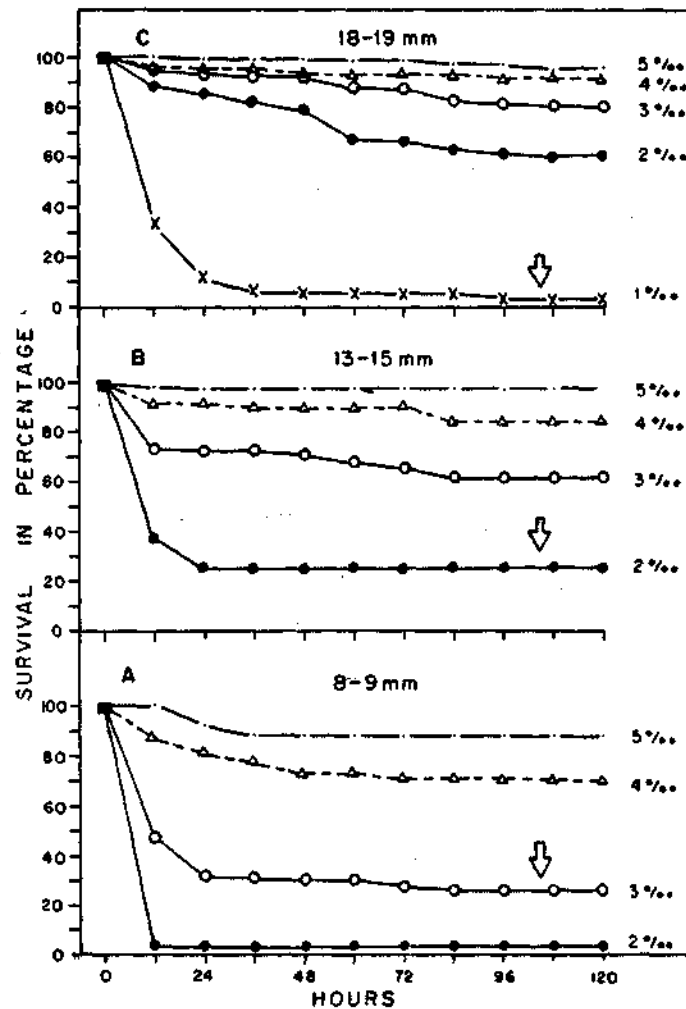


Fig. 3. Mean percentage survival of the 3 size groups of the postlarvae of *Penaeus indicus* in different test salinities below 5 ppt over different exposure periods. The arrows indicate the observed lethal salinities for the respective size groups.

as against 3 ppt in the case of the earlier size group. The post-larvae of 18-19 mm size showed good survival at 2 ppt salinity. The mean percentage survival at this salinity was 60% at the end of 120 hours of exposure indicating that it was within the tolerance range

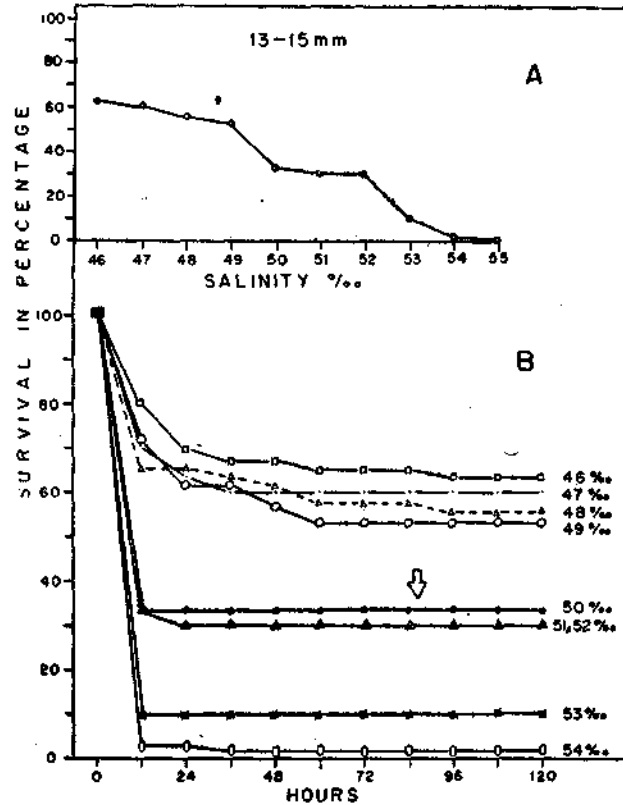


Fig. 4. Mean percentage survival of the postlarvae of *Penaeus indicus* (13-15 mm) in different test salinities: A. between 46 ppt and 55 ppt at 1 ppt interval at the end of 120 hours and B. above 46 ppt over different exposure periods. The arrow indicates observed lethal salinity.

of this group. In ppt the mortality was rapid and only 3.3% of the postlarvae survived at the end of the experiment. It would therefore appear that 1 ppt salinity is lethal to these advanced post-larval stages.



The highest salinity that can be considered as within the tolerating range for the size group 13–15 mm was found at 49 ppt in which 53% of the postlarvae survived at the end of 120 hours. A sudden drop in survival rate to 33% was noticed at 50 ppt which could therefore be considered as the lethal salinity for these sizes (Fig. 4).

The lower and upper mean lethal salinity levels estimated were respectively  $3.6282 \pm 0.09931$  and  $40.2268 \pm 0.56195$  ppt for 8–9 mm size,  $2.7812 \pm 0.10909$  and  $48.6191 \pm 0.28196$  ppt for 13–15 mm size and  $2.1071 \pm 0.06608$  and  $51.3134 \pm 0.31342$  ppt for 18–19 mm size postlarvae.

The salinity acclimation experiments carried out on 13–15 mm postlarvae, when subjected to gradual acclimation through 20, 15, 10, 5 and 3 ppt salinity levels, yielded 81.7% survival in the observed lethal salinity (2 ppt) at the end of 120 hours of exposure. When 18–19 mm postlarvae were subjected to salinities of decreasing and increasing strengths over a range of 0 ppt to 95 ppt more than 50% survival was recorded at a minimum salinity of 0.33 ppt and a maximum of 85 ppt at the end of 24 hours of exposure, indicating the possibility of a tolerating range between these levels. No postlarvae could withstand for more than 3 hours in 0 ppt salinity even after gradual acclimation process.

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# 6

## EFFECT OF SALINITY ON FOOD INTAKE, GROWTH, CONVERSION EFFICIENCY AND PROXIMATE COMPOSITION OF JUVENILE *PENAEUS INDICUS* H. MILNE EDWARDS

### *Introduction*

Salinity has been reported to have profound influence on survival, growth, distribution, food intake, conversion efficiency and proximate composition of a number of euryhaline organisms including the penaeid prawns.

### *Objectives*

The present study has been carried out to elucidate the effects of selected salinity levels on food intake, growth, conversion efficiency and proximate composition of juveniles of *Penaeus indicus* H. Milne Edwards as there was no information on these aspects.

Four sets of experiments were conducted; two of which were undertaken with a view to elucidate the effect of graded salinity levels on two size groups of juveniles [13 to 14 mm initial total length (1) and 26 to 32 mm initial total length (2)] of *Penaeus indicus*; while the third series was designed to derive a synoptic picture of food intake in hyper-saline waters (40‰ to 60‰) the last experiment was carried out for determining optimum feeding rates using three feeding levels and three salinity levels. The effect of salinity on the experimental animals was evaluated in terms of survival, quantity of food consumed, growth, conversion efficiency, protein efficiency ratio and deposition of nutrients in tissues.

### Material and methods

Juveniles of *Penaeus indicus* belonging to the same brood stock, raised at the Prawn Culture Laboratory of CMFRI at Narakkal were selected at random and maintained in water of salinity  $20 \pm 2\text{‰}$  for one week before transferring them to the test salinity levels. The salinity levels selected for the experiments 1 and 2 were 5‰, 10‰, 15‰, 20‰, 25‰, 30‰ and 35‰. For the third experiment salinity levels of 40‰, 45‰, 50‰, 55‰ and 60‰ were selected. In the fourth set of experiments three salinity levels and three feeding levels (10%, 20% and 30% of the initial body weight) were selected.

Food intake, conversion efficiency, relative growth and protein efficiency ratio were worked out for each experiment to study the influence of salinity on these aspects. Proximate analysis of feed and the experimental animals were determined using standard analytical procedures (AOAC).

The mean and standard deviation of the data were computed and analysis of variance (ANOVA) was performed to test the significance between treatments. Least significant difference method was used to test the significance between treatment means.

### Results

The results of the first two set of experiments showed that among the treatments, there was significant variation in the amount of food consumed ( $P < 0.05$ ). Maximum food intake was recorded at 25‰ in size group 1 (0.517 g) and at 20‰ in size group 2 (4.34). The minimum food intake was observed at 5‰ in both size groups. The food intake in each of the tested hypersaline levels (40–60‰) was approximately 10% of their initial body weight. Statistical analysis showed that there was no significant difference ( $P < 0.05$ ) in food intake among the tested hypersaline levels.

Experiment to study the effect of three feeding levels and three salinities showed that there was no significant variation in the amount of food consumed ( $P < 0.05$ ) at 5‰ level, amongst the feeding levels, and 10% feeding level recorded maximum food consumption. In 20‰ salinity, food intake at feed level 20% was significantly different ( $P < 0.05$ ) when compared to that of 10% and 30% feed levels. This indicates that feeding a daily ration of about 20% of the body weight is sufficient at this salinity level under similar environmental situations and thus wastage of feed can be eliminated.

At 35‰, no significant difference in food intake at the three feeding levels was observed and maximum food intake was about 10% of the initial body weight irrespective of the feeding levels and this may be optimum for this salinity, under the experimental environmental conditions.

Data on wet weight gain showed that in size group 1 maximum wet weight gain of 0.18 g was recorded at 25‰ and minimum (0.056 g) at 5‰ and the treatment means differed significantly ( $P < 0.05$ ). On the other hand in size group 2 maximum wet weight gain of 4.63 g at 20‰ and minimum of 1.46 g at 5‰ were obtained with significant difference ( $P < 0.05$ ) in the mean wet weight gain obtained among the treatments. Significant variation in dry weight gain and relative growth also was observed among treatments in both the experiments.

Conversion efficiency data showed maximum efficiency at 25‰ and minimum at 5‰ when food was given in excess in size group 1 indicating that nutrients of food was better utilised for synthesis of nutrients in the body at 25‰ by the animals. Similar results were obtained in size group 2, but the best efficiency was attained at 20‰. No marked variation was observed in the conversion efficiency with respect to feed levels (10%, 20% and 30% of the body weight) at salinity levels of 5‰ and 35‰. However, the conversion efficiency at 20‰ indicates better utilisation of food at feed level (20%) compared to that of other levels.

Protein efficiency ratio (wet weight basis) in size group 1 was not significant ( $P < 0.05$ ) among treatments, however it was fairly higher (0.86) at 25‰ than at 5‰ (0.666) salinities. The maximum protein efficiency ratio (PER) at 25‰ coincides with the maximum food intake. In size group 2 maximum PER was recorded at 20‰.

A steady decline in the water content of the experimental prawns was observed from lower salinity (5‰) to higher salinity (35‰) treatments and the treatment means differed significantly ( $P < 0.05$ ). Maximum water content of 72-73% and minimum of 66% were recorded in size group 1 and 2. In the fourth experiment there was not much variation in the moisture content amongst the different feeding levels in the same salinity treatment groups, but a decreasing trend was observed as the salinity increased from 5‰ to 35‰.

In both the size groups protein content showed significant rise as the salinity increased from 5‰ to 25‰ and 5‰ to 20‰ respec-

tively and thereafter it declined. The maximum recorded protein content was 71.95% for size group 1 and 73% for size group 2 at 25‰ and 20‰ respectively, while minimum of 64% at 5‰ in size group 1 and 2 respectively which were statistically significant ( $P < 0.05$ ). The results of the fourth set of experiments showed that at lower (5‰) and higher (35‰) salinity levels accumulation of protein was poor while at 20‰ the protein deposition was maximum (72%). There was again variation in the protein content of the tissue in relation to different feeding levels at various salinity levels.

The maximum lipid contents of tissues observed during the study were 17.76% and 14.91% at 10‰ and 5‰ respectively in groups 1 and 2; and lowest being 12.67%, 10.67% at 25‰ and 20‰ respectively, thus showing the greatest variation in the lipid levels. Results of the fourth experiment showed that there was no marked difference in lipid content within the same salinity at different feeding levels, but lipid content differed at the tested salinities. The lowest value of lipid was recorded at 20‰ and highest at 35‰.

### Conclusion

The results of the present investigation show that salinity has profound influence on the food consumption, growth, conversion efficiency and proximate composition of the tissues of the euryhaline penaeid prawn *Penaeus indicus*. It is apparent that tolerance to salinity is age dependent. The data on food intake, growth and conversion efficiency and body composition show that size group 1 requires on optimum salinity of about 25‰ and the size group 2 about 20‰. The food intake was independent of the quantity of food supplied but was dependent on the salinity of the media. The maximum food consumption, the best conversion efficiency ratio and protein efficiency ratio at 25 ppt and 20 ppt for size group 1 and 2 respectively may be attributed to the fact that these salinities may be nearer to the optimum and that they are perhaps least disturbed and possibly under least stress at these salinity levels.

The minimum growth at 5 ppt in both size groups 1 and 2 may be related to lower metabolic rate at that salinity and least food intake. Increased ammonia excretion rate was recorded in lower (5‰) and higher salinity levels (35‰) indicating increased catabolism of amino acids at these salinity levels. It appears that considerable amount of energy derived from ingested food was spent in excretion rather than tissue building.

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# 7

## COMPARATIVE STUDIES ON PROTEIN, CARBOHYDRATES AND FAT CONTENTS IN *PENAEUS INDICUS* DURING OVARIAN MATURATION IN NATURE AND INDUCED MATURATION EXPERIMENTS

### *Introduction*

For an understanding of the reproductive physiology of the prawns, especially in the context of the application of the new techniques like eyestalk ablation, for induced maturation of the gonads, biochemical changes, correlated with the nutritional aspects and reserves of the prawn during the reproductive cycle, taking place in the gonads and other organs like the hepatopancreas and muscles have to be monitored properly. So the changes in the biochemical parameters such as the levels of proteins, carbohydrates and lipids in different forms in the Indian white prawn *Penaeus indicus*, have been studied.

### *Material and methods*

The prawns for the present study were collected from the ponds of Narakkal Prawn Culture Laboratory. The prawns were about the same size (carapace length  $3.9 \pm 0.2$  cm). All the prawns used were in intermoult condition. Unilateral eyestalk ablation was performed using an electrocauteriser. Within 5-10 days the prawns attained full maturity and prawns at different stages of maturity were collected during the maturation. Spent stages were obtained after the prawns had spawned in the spawning tanks. The stages of maturity were identified according to Rao (1968, *FAO Fish. Rep.*, **57** (2) : 285-301).

For biochemical analysis, the different tissues namely, hepatopancreas, first abdominal segment muscle and ovary were separated from the fresh specimen at different stages of maturity of the ovaries. The wet weight of the tissue were taken and oven dried at 70°C for 48 hrs, thereafter kept in a dessicator till a constant weight was attained. The gonadosomatic index was determined for each stage of development using the formula given by Farmanfarman *et al.* (1958, *J. exp. Zool.*, 138 : 355 - 367).

For the above mentioned three tissues, the biochemical parameters estimated were protein, non-protein nitrogen, total free amino acids, oligosaccharides, polysaccharides and total lipids. All the values are expressed in mg/g wet weight.

Protein was estimated by Micro-Kjeldahl method by precipitating the protein in 10% tri-chloroacetic acid. The supernatant was used for non-protein nitrogen estimation. Lipids were estimated according to Kates (1969, *Laboratory techniques in biochemistry and molecular biology*, 3), oligo-saccharides and polysaccharides according to Johnson and Davies (1972, *Comp. Biochem. Physiol.*, 418 : 433 - 455) and total aminoacids was estimated according to Lee and Takahshi (1966, *Analyt. Biochem.*, 14 : 71 - 77).

#### Results and discussions

The significant points emerging from the study are as follows:

1. In the Indian white prawn *Penaeus indicus*, after eyestalk ablation, there is a significant increase in the weight of the ovary, as indicated by the increase in the gonado-somatic index of the animal (Fig. 1).
2. The gonado-somatic index of *P. indicus* collected from the wild population always showed lesser value than that of the immature cultured specimens.
3. The lipid content of cultured, immature *P. indicus* was higher than that of the wild specimens caught from the sea. The other biochemical parameters did not show any marked difference in the two categories.
4. Proteins formed one of the major constituents comprising 13.7% wet weight and 43.5% dry weight. Similar results were obtained by Lawrence *et al.* (1979) in the case of *P. vannamei* (43%), *P. stylirostrus* (37.3%) and *P. setiferus*

(42.3%), and by Pillai and Nair (1973) in *Metapenaeus affinis* (41–43%). There is an increase of lipids from  $45.33 \pm 3.54$  in stage I,  $38.61 \pm 2.03$  mg/g wet weight in stage II to  $53.27 \pm 1.84$  in stage III and  $74.85 \pm 1.72$  mg/g wet weight in stage IV indicating a major build up after stage II. On spawning the lipid level drops down

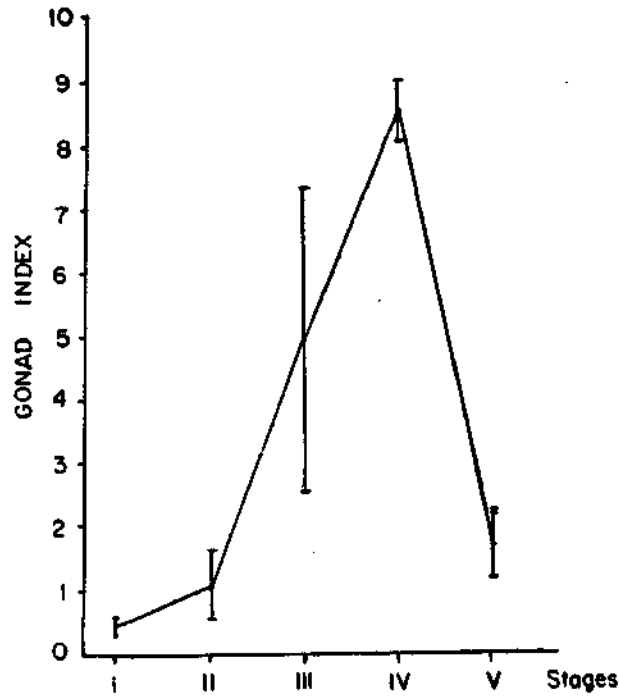


Fig. 1. Changes in gonadosomatic index of females of *P. indicus* with different stages of maturity.

to that of stage I. The rise in the protein and lipid level and a simultaneous decline in polysaccharide fraction (after stage II) of the ovaries of ablated prawns might indicate that the ovarian polysaccharides may be used up for the making of protein or lipid yolk synthesis (Fig. 2).

5. The lipid level of the hepatopancreas showed a decline with advancement of maturity, while no variation was recorded



in the control. This may be due to utilisation of resources for vitellogenesis. This has also been shown by Adiyodi and Adiyodi (1970 a, *Indian J. Biol.*, 8 : 222-223; 1972, *Biol. Bull.*, 143 (3): 354-369) who suggested the possibility of utilisation of hepatopancreatic unsaturated fatty acid and phospholipid by the ovary for vitellogenic purpose.

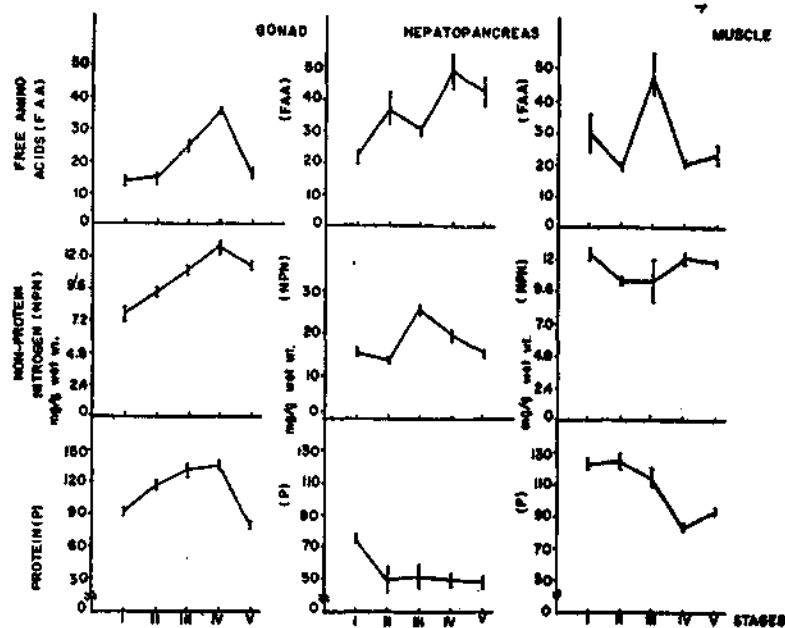


Fig. 2. Levels of protein, non-protein nitrogen and free aminoacids in the gonad, hepatopancreas and muscles of *P. indicus* during ovarian cycle. Values are mean  $\pm$  SD.

The utilisation of hepatopancreatic sugars for yolk synthesis has been reported by earlier workers. Adiyodi and Adiyodi (1970 a, *Indian J. Biol.*, 8 : 222-223) suggested a cyclic fluctuation in the mono and disaccharides in the hepatopancreas of *P. hydromus* in relation with the ovarian cycle.

In the present study, the polysaccharides showed a fluctuation and so did the oligosaccharides. It is interesting to

note that the increase in the former and simultaneous decrease in oligosaccharides in the stages is evident. Pillai and Nair (1973, *Mar. Biol.*, 18: 167-198) suggested a possible transfer of glycogen from the hepatopancreas to the ovaries during the breeding season in the crab *Uca annulipes*. As the molecules of glycogen are comparatively bigger, its conversion to smaller units like glucose before its entry into the haemolymph may be needed. This might perhaps be the reason for the reciprocal relationship that was observed in the oligo and polysaccharide content in the hepatopancreas of the prawn (Fig. 3).

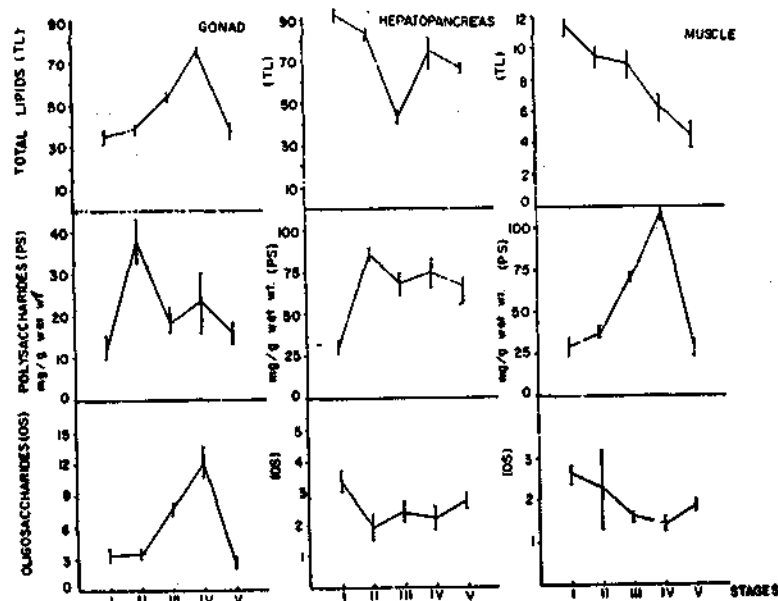


Fig. 3. Levels of oligosaccharides, polysaccharides and total lipids in the gonad, hepatopancreas and muscles of *P. indicus* during ovarian cycle. Values are mean  $\pm$  SD.

The protein content in the hepatopancreas is very low. In fact the percentage of non-protein nitrogen fraction was always found to be higher than the percentage of protein nitrogen. A steady increase in the content of free amino acids is seen in the hepatopancreas up to stage IV and then a decline. This

increase in the free amino acid content in the hepatopancreas may be due to protein hydrolysis, the origin of the hepatopancreatic free amino acids. The increase in the hepatopancreatic free amino acids can also occur through the absorption from the gut or even by the biosynthesis of amino acids from carbohydrates as reported in *Homarus*, *Astacus* and *Carcinus* (Schoffeniels and Gilles, 1970, In: *Chemical Zoology*, Academic Press).

6. The muscle of the prawn is characterised by the high content of the proteins and low level of lipids in comparison with ovary and hepatopancreas. A decline in the content of protein observed after stage II, reaching the lowest value at stage IV is probably due to the muscle protein being used up towards the development of the ovary. In the wild population, the gonadal maturity is a long drawn out process, but in the eye-stalk ablated prawns, the whole process is hastened. Hence it is possible that some nutrients from the body itself may be observed and thus show a sign of decrease. However, in crustaceans no such phenomenon has been reported earlier, although in other experimental animals from other phyla, there are several instances recorded on decline in muscle proteins at the time of ovarian maturation.

As in the case of proteins, lipids also did show a similar trend, small amounts of lipids being used for meeting the metabolic demand of the animal during the ovarian cycle.

An increase in the level of polysaccharides is observed in the muscle of *P. indica*. In the immature stage of prawn, it is only  $29.2 \pm 10.5$  mg/g wet weight. In the subsequent stages it increases to  $37.06 \pm 4.02$ ,  $69.6 \pm 1.81$  and reaching  $111.89 \pm 5.67$  mg/g wet weight in the fully matured stage. After spawning there is a decline to 27.24. Similar observations were made by Rangnekar and Madhyasha (1972, *Indian J. exp. Biol.*, 9: 462-464) in *M. monoceros* where the glycogen content in the hepatopancreas was reduced with simultaneous increase in the muscle. They suggested that excess sugars obtained through glucogenolysis in the hepatopancreas may be mobilized for the synthesis of glycogen in the muscle tissue (Fig. 3).

7. In conclusion it may be mentioned that as the ovary develops and vitellogenesis sets in, the organic reserve also built up by the gradual accumulation of protein, non-protein nitrogen

and lipids, free amino acids and carbohydrates. As protein and lipid are the major energy giving biochemical compounds of yolk, large accumulation is seen in the ovary. The hepatopancreas, a marvellous storage organ in the prawn, show a decline in the organic resources as the gonadial maturity sets in. This probably is due to the mobilization of reserves especially lipids, towards the development of the ovary. The muscle of the prawn is relatively stable when compared with the ovary and hepatopancreas.

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## NUTRITIONAL REQUIREMENTS OF POSTLARVAE OF THE PRAWN *PENAEUS INDICUS*

### *Introduction*

Hatchery production of prawn seed involves two important phases; one is the rearing of early larval stages in the hatchery and the other is further rearing of postlarvae in nursery until they are stocked in growout ponds. Availability of nutritionally well balanced compounded feed would greatly simplify the production of prawn seed. Proper feeding and management of postlarvae in the nursery are crucial for higher rates of survival and faster growth.

### *Objectives*

With the objective of finding out the nutritional requirements, with special emphasis on protein requirement, of postlarvae of the prawn *Penaeus indicus* and to use the information for evolving suitable compounded feeds using low-cost ingredients, these investigations were carried out.

### *Material and methods*

The postlarvae (PL) were divided into three groups according to their age in days from the day of becoming postlarvae — PL 1 to PL 10 was taken as group I, PL 11 to PL 25 as group II and PL 27 to PL 42 as group III. Two series of experiments were conducted using purified and semipurified diets. The former diets were used for studying the nutritional requirements and the latter diets were

used to explore the possibilities of rearing the postlarvae on compounded diets.

Six isocaloric purified diets were formulated, using casein, starch, lipid (sardine oil and groundnut oil in the ratio 1:1), cholesterol, vitamins, minerals and cellulose. The protein content of diets was increased by 10% from 20 to 70% by varying the casein content. The energy was made up using carbohydrate (starch). Six semipurified diets were formulated using a protein base (consisting of prawn waste, mantis shrimp (*squilla* spp.), groundnut cake and fishmeal), casein, groundnut oil, vitamins, minerals and tapioca powder. The protein content of the diets were 20.23%, 32.12%, 42.07%, 50.81%, 60.77% and 70.69% and each diet was prepared as granules of 300, 400 and 500 microns size.

Feeding experiments were carried out in 5 litre capacity glass containers. Postlarvae of *P. indicus* belonging to a single brood were taken and randomly selected, stocked in the containers consisting of four litres of filtered sea water. Twenty postlarvae with an average length and dry weight of 5 mm and 0.057 mg respectively were stocked in each container in the case of group I postlarvae. Whereas group II and group III with average length and weight of 9.96 mm, 0.89 mg and 13.14 mm, 3.46 mg respectively were stocked at the rate of 10 animals per container. Three replicates were kept for each treatment. The first group of postlarvae were fed with 300 micron granules and group II and III were fed with 400 and 500 micron granules respectively. The rate of feeding was 100 mg, 150 mg and 200 mg per day per container for the three groups of postlarvae respectively. The larvae were fed twice daily in divided doses and water was completely changed every day.

Feeding experiments with semipurified diets were conducted in 2 litre capacity glass containers, stocking at the rate of 10 animals per container with two replicates for each treatment. The rate of feeding was 30, 50 and 100 mg per day per container respectively, in two divided doses. The water management was similar to that of the first experiment. In these experiments rotifers (*Brachionus plicatilis*) and cladocerans (*Moina* spp.) were fed as control feed for comparison. To group I postlarvae rotifers were fed for the first five days at the rate of 20,000 per container and subsequent five days *Moina* spp. was fed at the rate of 1,000 per containers. For group II and III postlarvae only *Moina* spp. was fed at the rate of 1500 and 2000 respectively per day per container. The salinity of water used for group I postlarvae was  $32 \pm 1$  ‰ and for group II and III it was  $20 \pm 1$  ‰. The temperature varied from 27.6 to 31.3°C and pH ranged between 7.6 and 7.9.

## Results

The relation between growth of different groups of postlarvae and dietary protein level was shown in Fig. 1. The growth of group I

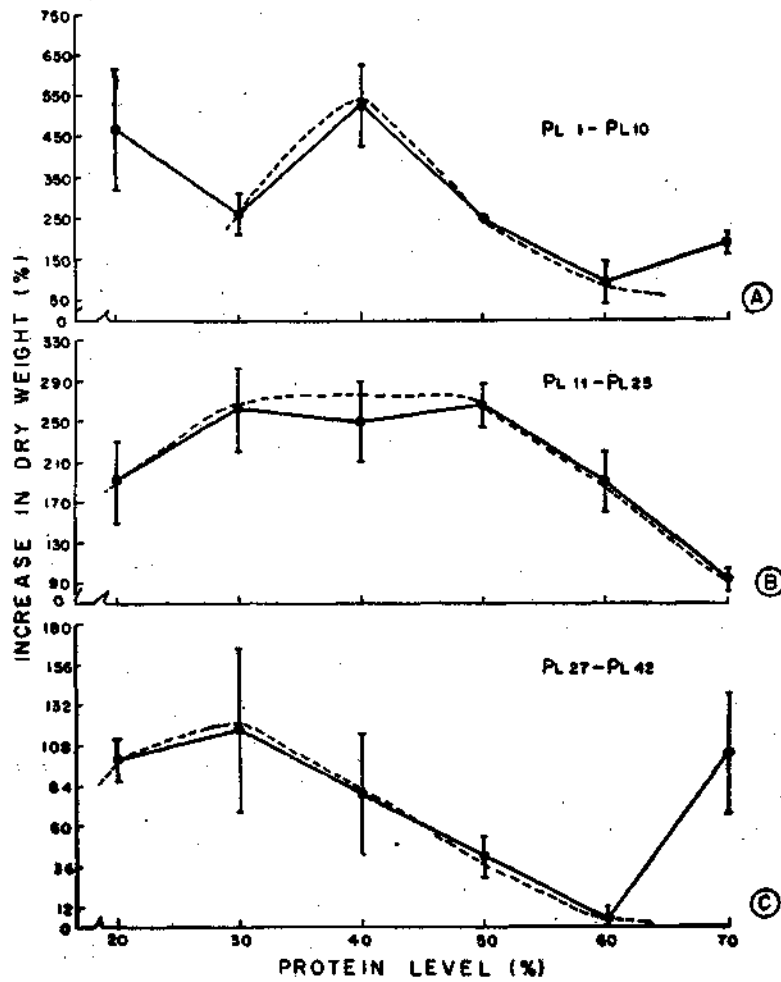


Fig. 1. Relationship between dietary protein level and growth in dry weight of postlarvae.

postlarvae, both in length and weight, increased with the increase in successive increase in dietary protein level upto 40% and declined

there after (Fig. 1 curve A). The growth obtained by the diet with 40% protein was highly significant ( $P < 0.01$ ) from that of the other diets. The growth curve of group II postlarvae (Fig. 1 curve B) had shown a steep increase in the growth upto 30% with a slight decline at 40% level and again it was elevated at 50% protein approximately to the level of 30% protein. Thereafter there was gradual decline in the growth with increase in dietary protein level.

The growth of group III postlarvae increased with dietary protein level upto 30% and declined with higher protein levels. The growth recorded by the diet with 30% protein, was found to be significantly higher ( $P < 0.05$ ) from that of the other diets.

With semipurified diets, the postlarvae could be successfully reared and the results were comparable to that of the live feeds, rotifers and cladocerans. Group I postlarvae grew faster on the semi-purified diet with 50.81% protein, along with 20% carbohydrate and 10.4% lipid. However, the rate of survival in the case of live feeds was slightly higher (95%) compared to that of the semi-purified diet (75%). In the case of group II and III postlarvae the diet with 32.12% protein in combination with 35% carbohydrate and 10.8% lipid gave the highest growth and survival (70 and 85% respectively) which was superior to the growth and survival obtained by the control live feed.

#### Conclusions

The protein requirement in the diets for the postlarvae of *P. indicus* decreases with the increase in their size. The protein requirement in the diets for group I postlarvae (PL 1 to PL 10) was found to be 40% in combination with 35% to 40% carbohydrate and 12% lipid. For group II and III (PL 11 to PL 25 and PL 27 to PL 42) postlarvae it was 30% in combination with 35% to 40% carbohydrate and 10% lipid.

Postlarvae could be successfully reared using compounded feeds upto stocking size, and the growth and survival obtained by compounded feeds was superior to that of the control feeds rotifer and *Moina* spp.

#### Paper under publication based on this dissertation

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**STUDIES ON THE USE OF GROWTH PROMOTING  
AGENTS IN THE DIETS OF  
*PENAEUS INDICUS* H. MILNE EDWARDS**

*Introduction*

The need to evolve appropriate compounded feed has well been recognised and it is identified as the largest single input in culture systems. This has lead to constant effort to develop compounded feeds of higher efficiency which stimulate faster growth, in order to make shrimp culture an economically viable proposition. There are certain chemical and biological substances called growth promoting agents, which promote faster growth when incorporated in the feed in small quantities. Some of the growth promoting agents are successfully used in commercial animal feeds.

*Objectives*

The present study has been undertaken to explore the possibilities of using some of the selected substances such as hormones, antibiotics, glucosamine, chitin and alfalfa extract (plant material) as growth promoting agents in the diets of *Penaeus indicus* in order to achieve faster growth and better food conversion efficiency.

*Material and methods*

The substances tested for their growth promoting property were oxytetracycline (antibiotic), orabolin (ethylo-estrenol, synthetic steroid hormone), thyroid (hormone), alfalfa extract lucern,

plant material), glucosamine (chemical) chitin (crustacean shell material) and testosterone (hormone). Except chitin, all the materials were commercial products and were purchased from the market. Chitin was prepared in the laboratory from prawn shell by the method described by Madhavan and Ramachandran Nair (1975, *Fish Tech.*, 12 (1): 81-82). Dried prawn shell was crushed and defatted first using methanol-chloroform (1:2 ratio) mixture. The residue was boiled with 4% sodium hydroxide twice and washed thoroughly and boiled with distilled water, dried and powdered.

#### *Formulation and preparation of diets*

##### *Set 1*

For the purpose of studying the effect of these substances, a basal purified diet based on the formula of Kanazawa *et al.* (1970, *Bull. Jap. Soc. Sci. Fish.*, 36: 949-954), with suitable modifications was formulated. The diet consisted of casein (40%), Cod liver oil (5%), sucrose (10%), starch (30%), vitamins and minerals (3%), along with other additives. Agar agar (3%) was used as the binding agent. To this basal diet, each substance was incorporated at a definite level at which some of these were used in fish diets in the literature. Thus, seven diets D0, D1, D2, D3, D4, D5 and D6 were prepared. D0 was the control diet without any growth promoter. The diets D1 to D6 consisted of oxytetracycline (10 mg), orabolin (0.5 mg), thyroid (1.0 mg), alfalfa extract (2 ml), glucosamine (0.8 g), chitin (0.8 g) and testosterone (2.5 mg) per 100 g diet respectively.

##### *Set 2*

The second set of diets was designed to study the effect of different levels of alfalfa extract, glucosamine, chitin and testosterone, on the growth of *Penaeus indicus* which showed positive growth response in the first set of diets. To the same basal diet each of the four substances was incorporated at four different levels. Alfalfa extract was incorporated at 0.5, 1.0, 2.0 and 3.0 ml/100g diet and the diets were designated as D8, D9, D10 and D11. Glucosamine was used at 0.2, 0.5, 0.8 and 1.5 g/100 g diet and the resultant diets were D12, D13, D14 and D15. Whereas the four diets containing chitin at 0.2, 0.5, 0.8 and 1.5 g/100 g diet were D16, D17, D18 and D19. Finally testosterone was used at 1.0, 2.5, 3.5 and 5.0 mg/100 g diet and these were designated as D20, D21, D22 and D23.

### Set 3

The third set of diets was aimed at to study the synergistic effect of these selected growth promoters on the growth and food conversion ratio of *Penaeus indicus*. Accordingly, seven different diets designated as D24, D25, D26, D27, D28, D29 and D30 were formulated with seven different permutations and combinations of alfalfa extract-glucosamine, alfalfa extract-chitin, alfalfa extract-testosterone, chitin-testosterone and all the four substances together respectively. Each substance was incorporated at the optimum level indicated in the second set.

### Feeding experiments

Feeding experiments were conducted with the diets on the juveniles of *Penaeus indicus* obtained from the hatchery. The animals with an average length of 20 mm and average live weight of 35 mg were randomly selected and stocked in 25 litre capacity circular plastic troughs containing filtered sea water with a salinity of  $17 \pm 1$ ‰. In each trough eight animals were kept with three replicates for each treatment. The animals were fed a weighed quantity of food *ad libitum*. The left-over food was collected, sediments removed daily and water was changed once in 3 days. The duration of feeding experiment was for four weeks.

### Results

The results of the first experiment are shown in Fig. 1. The diets supplemented with alfalfa extract, glucosamine, chitin and testosterone had produced superior growth food conversion ratio compared to the control diet. Where as the growth and food conversion ratio obtained by the diets supplemented with oxytetracycline, thyroid and orabolin was similar to that of the control diet. The data when analysed by the method of Least Significance difference, was found that the growth and food conversion ratio obtained by these diets were significantly different ( $P < 0.05$ ) from that of the control diet. Among the candidate growth promoting agents, which showed positive response, the diet with testosterone gave the highest increment in length (80.76%), dry weight (473.83%) and best food conversion ratio (1.61%) compared to the growth (38.58% in length, 268.69% in dry weight) and food conversion ratio (2.84) obtained by the control diet. This was followed by the diets with glucosamine, chitin and alfalfa extract in the descending order. The rate of survival was also significantly higher ( $P < 0.05$ ) in the case of testosterone (87.5%) compared to that of the other diets. The

body protein of the animals fed with the diets having testosterone, orabolin, thyroid, alfalfa extract, was higher than that of the animals fed with the control diet, whereas it was slightly lower in the case of animals fed with the diet with glucosamine and testosterone.

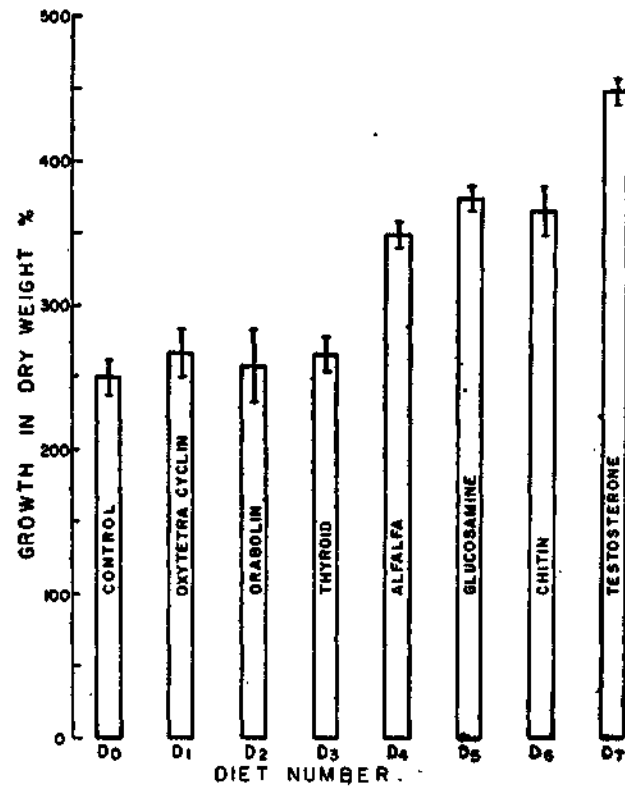


Fig. 1. Relationship between growth and the diets with different growth promoting agents.

#### Experiment 2

The relationship between the growth and dietary levels of alfalfa extract is shown in Fig. 2. The growth of prawns increased as the dietary level of alfalfa extract increased from 0.5 to 1.0 mg/100 g diet and the growth rate declines with higher levels of it. The food conversion ratio and rate of survival improved upto 2.0 ml/100g diet. The growth obtained by the diet with 1.0 ml/100 g diet of alfalfa extract was significantly higher ( $P < 0.05$ ) from that of the

other diets. Using polynovial regression curve (dotted line), it was found that the optimum level of alfalfa extract in the diet, for optimum growth, food conversion ratio and survival, was found to be 1.76 ml/100 g diet.

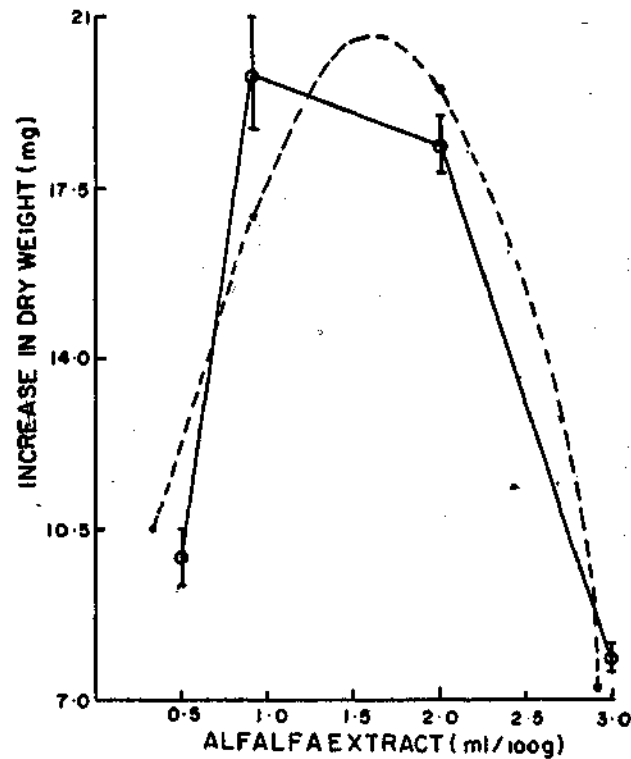


Fig. 2. Relationship between growth and dietary alfalfa extract.

In the case of glucosamine, the growth (Fig. 3), survival and food conversion ratio increased and improved with the increase in the dietary level of glucosamine upto 0.8 g/100 g diet. Higher levels of it suppressed growth. The results obtained with the diet having 0.8 g glucosamine per 100 g diet were significantly superior ( $P < 0.05$ ) to the other glucosamine levels. The optimum level of glucosamine in the diet was found to be about 0.98 g/100 g diet for obtaining optimum growth and food conversion ratio (dotted line).

It was found that in the case of chitin there was linear relationship between the growth of prawns and the dietary chitin level (Fig. 4). The growth was significantly higher ( $P < 0.05$ ) at 1.5 g/100g diet level.

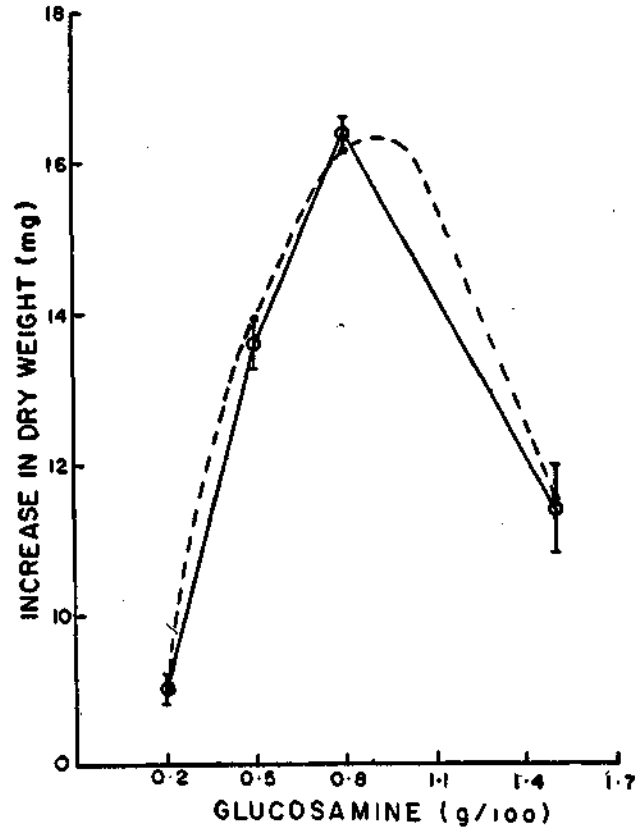


Fig. 3. Relationship between growth and dietary glucosamine level.

The growth and survival of prawns progressively increase (Fig. 5) with increase in the dietary testosterone level from 1.0 to 5.0 mg/100 g. The food conversion ratio also improve linearly. The growth and food conversion ratio were significantly higher ( $P < 0.05$ ) at 0.5 mg level.

### Experiment 3

Among the different combination of growth promoting agents tested, the diet containing testosterone-chitin gave the highest growth survival and best food conversion ratio. This was followed by the

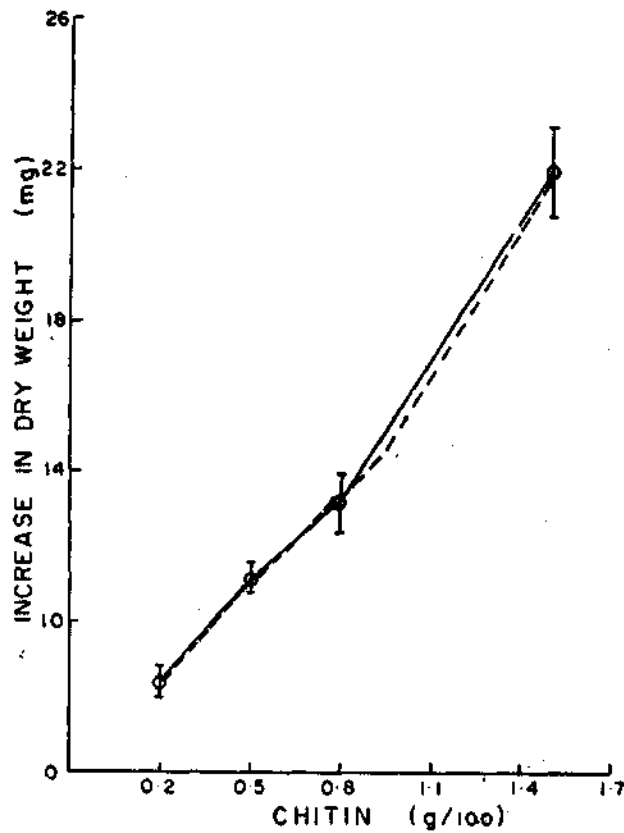


Fig. 4. Relationship between growth and dietary chitin level.

diets with glucosamine-alfalfa extract. The synergistic effect was seen in the food conversion ratios obtained by the seven combinations, which were better than those obtained when they were used individually.

### Conclusions

Testosterone, glucosamine, chitin and alfalfa extract when supplemented in the diet at 5 mg, 0.98 g, 1.5 g and; 1.5 ml/100 g

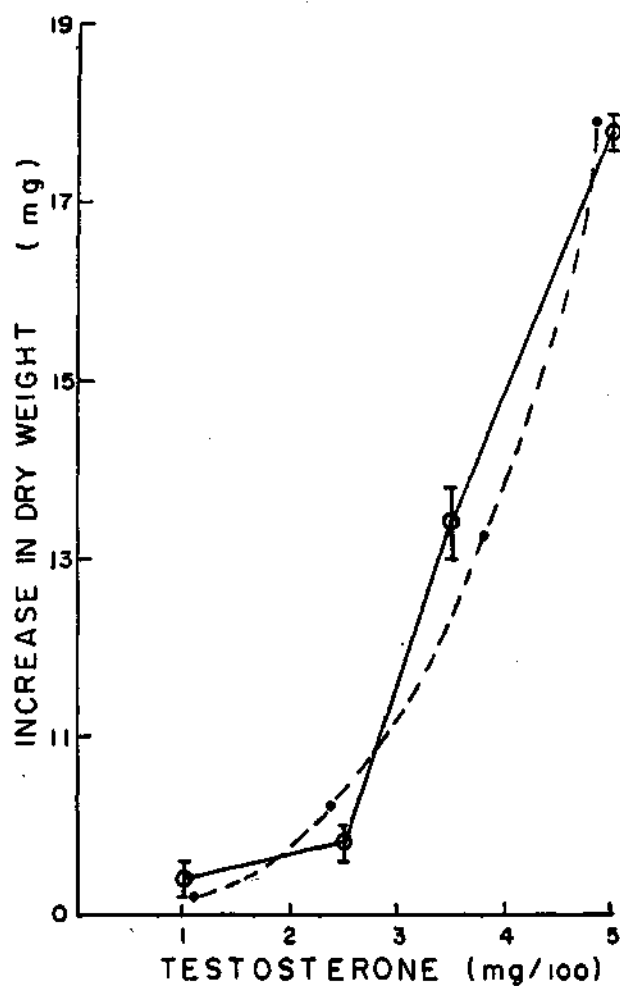


Fig. 5. Relationship between growth and dietary testosterone level.

respectively enhanced growth and improved food conversion ratio in *Penaeus indicus* and can be considered as growth promoters.



Testosterone and chitin were found to have a synergistic effect as growth promoters when used in combination at 5 mg and 1.5 g/100 g respectively.

Only tetracycline, orabolin and thyroid failed to give enhanced growth in *P. indicus* at levels used in the experimental diets.

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# 10

## FOOD VALUE OF ROTIFER, BRINE SHRIMP AND *MOINA* TO POSTLARVAE OF *PENAEUS INDICUS* H. MILNE EDWARDS REARED IN THE LABORATORY

### *Objectives*

The objective of the investigation was to find out which is advantageous as live feed for growing the postlarvae of *Penaeus indicus*, among *Moina* rotifer, nauplii and decapsulated cysts of brine shrimp. In the studies postlarvae of 1-10; 13-20; 21-30 and 35-50 day stages were used. The larvae used in each set of experiments were from the same brood. Quadruplicates were maintained in each experiment.

### *Material and methods*

*Moina* sp. and rotifer *Brachionus plicatilis* were grown in the laboratory by feeding them with algae mainly of *Chlorella* sp. induced to bloom by enriching the brackishwater with groundnut oil cake. Okhamandal, Gujarat and Tuticorin (Tamil Nadu) strains of brine shrimp *Artemia salina* cysts were used and fed as decapsulated cysts and freshly hatched nauplii. The hatching of *Artemia* cysts were carried out at 26.5-27.5°C. 'Okhamandal cysts' hatched within 30 hrs, while 'Tuticorin cysts' failed to hatch out and so only nauplii of 'Okhamandal cyst' were used. The technique of decapsulation followed was essentially after Sorgeloos, in which the dark brown coloured external shell, chorion was removed in four stages. 250 mg of cyst was hydrated in 500 ml of filtered sea water with continuous aeration for 1½ hrs. Then the cysts were

seived with 100 micron mesh sieves, washed with filtered sea water and transferred into a 100 ml beaker. To this, a mixture of 40 ml active sodium hypochlorite and 20 ml filtered sea water was added and mixed. Then 1.5 ml of 40% sodium hydroxide was added slowly along the wall of the beaker and mixed thoroughly. Although care was taken to keep the temperature below 40°C by immersing the beaker in ice water. After 7 to 10 minutes the change of colour from brown to orange indicates solubilisation of chorion. The decapsulated cysts thus obtained were washed thoroughly with filtered seawater by keeping the cysts in nylon bolting silk cloth till the smell of chlorine disappeared. Then to the cysts having 200 ml of sea water, 0.5 ml. of 1% sodium thiosulphate was added to remove residual chlorine, stirred and after 10 minutes the decapsulated cysts were transferred to a separating funnel and aerated. Now while the cysts sink, other organic matter float and the cysts transferred to a nylon meshed bag, washed repeatedly, counted, weighed, filled in glass vials and stored in a refrigerator. While hydrated, it was observed that 'Okhamandal strain' floated, 'Tuticorin cysts' sunk to the bottom of the beaker. In all cases appropriate daily rations were sorted, counted placed in glass vials, stored in a freezer and used within three days.

TABLE 1. *Experimental conditions in the four set of experiments*

Experiment	size	Number in 3.5 lit	pH	Tempera- ture (°C)	Oxygen (ml/l)	Salinity (‰)
1 PL 1-10	5.0	15	8.14±0.25	27.5±1.1	3.83	28.03±0.15
2 PL 13-20	7.6-8.6	10	8.18±0.08	27.5±0.5	3.55	24.90±0.13
3 PL 21-30	13.8-15.2	8	8.24±0.11	27.0±1.2	3.58	19.88±0.17
4 PL 35-50	16.5-17.2	28	8.62±0.23	27.2±1.3	3.58	20.01±0.18

TABLE 2. *Feeding level/day/beaker (5L)*

Experiment	<i>Moina</i>	rotifer	<i>Artemia</i> nauplii	Decapsulated cyst	
				Okhamandal strain (mg)	Tuticorin strain (mg)
1 PL 1-10	3,330	40,000	2,325	18.63	—
2 PL 13-20	3,625	40,000	3,625	21.99	21.10
3 PL 21-30	5,900	56,000	6,700	39.84	42.31
4 PL 35-50	17,400	1,00,000	8,600	88.21	—

Experiments were conducted in 5 litre glass beakers having 3.5 litre filtered brackishwater. The feeding was carried out *ad libitum* twice daily at 1000 and 2000 hrs, the leftover and the faecal matter removed after 6 hrs of feeding (Table 1 & 2).

## Results

The biochemical composition of the different feeds are given in Table 3. Analysis of variance showed no significant difference ( $P < 0.05$ ) in survival for all stages except in PL 35-50. Postlarvae fed on rotifer showed least survival (79%). In all cases growth was high in postlarvae fed with decapsulated *Artemia* cyst. In contrast, the rotifer *Brachionus plicatilis* showed poor increase in length and weight. In PL 1-10 no significant difference in percentage length increase was noted between postlarvae fed with rotifer,

TABLE 3. Proximate composition of the live feeds

	<i>Moina</i>	Rotifer	<i>Artemia</i> nauplii (Okhamandal strain)	Decapsulated <i>Artemia</i> cyst Okhamandal strain	Tuticorin strain
Protein (%)	58.50	56.32	60.12	61.54	58.04
Lipid (%)	21.42	21.21	20.20	22.20	27.14
Carbohydrate(%)	7.75	7.26	8.06	9.93	9.92
Ash (%)	10.45	13.04	9.81	5.80	4.22
Calorific value (K cal/g ash free dry wt.)	5.65	5.48	5.64	5.96	6.25

*Moina* and between those fed with *Artemia* nauplii and decapsulated cyst. But a difference in dry weight ( $P < 0.05$ ) was noted in all cases, with postlarvae fed on decapsulated cyst superseding all the other feeds in percentage weight increase. In PL 13-20, no difference in length was observed between the postlarvae fed with *Artemia* nauplii and *Moina*, but a better dry weight increase was noted in those fed with *Artemia* nauplii. Among the two strains of *Artemia* cysts 'Tuticorin strain' was found better than 'Okhamandal strain' and statistically significant growth was observed in postlarvae (PL 13-20), the 'Okhamandal strain' gave a better dry weight increase in PL 21-30. The other feeds used gave poor growth rate compared to decapsulated cyst. *Artemia* nauplii fed larvae showed better length, dry weight and protein increase than *Moina* and rotifer in early postlarvae, while those fed on rotifer fared badly. In 35-50 too, decapsulated cyst gave pronounced growth followed by *Artemia* nauplii.

Though increase in protein was significant ( $P < 0.05$ ) in all cases, postlarvae fed with decapsulated cysts showed a higher increase. The level of increase was also significant among the large PL13-20

and 21-30. Postlarvae fed on rotifer consistently showed poor increase in protein level while those fed with *Artemia* nauplii showed a better incorporation of protein than *Moina*.

Particle size of rotifer, *Moina*, *Artemia* nauplii were respectively 173, 780, and 484  $\mu\text{m}$  and did not seem to influence survival in all stages except possibly in PL 35-50. Herein it appears that the smaller size of rotifer and *Artemia* nauplii could be a factor that has reduced the chances of the larvae picking them up. Although the decapsulated cysts were too small, they were picked up with less difficulty, because the cysts sediment to the bottom and thereby making them easily accessible. Here too, the light orange colour of the decapsulated cysts might have attracted the postlarvae.

Moulting frequency, colour and chromatophore development coupled with the activity of the larvae were better in those fed with decapsulated *Artemia* cyst. Among the decapsulated cysts the 'Tuticorin strain' having higher lipid content fared better than the other, though its hatching efficiency was poor, whereby showing that hatching efficiency of *Artemia* cyst has no direct relationship with the food value of the cyst.

#### Conclusion

In the present study it is evident that although all the postlarval groups fed with live feed gave a comparable result in terms of survival, none of them showed a better growth rate than those fed with decapsulated *Artemia* cyst. All the live feed used have optimal protein: Carbohydrate ratio, wherein protein and carbohydrate being above optimal level, lipid content has been found to be the key factor in enhancing the growth rate of prawn postlarvae.

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# 11

## FOOD AVAILABILITY AND SELECTIVITY IN PRAWN CULTURE POND

### *Objectives*

In view of the importance of intensive culture of prawns in natural ponds and paddy fields a proper understanding of the food requirements and selectivity of these species cultured in these environments in relation to the food availability in the ponds, is quite essential. With this view, an attempt has been made to study the selectivity of food organisms, if any, by prawns in a few selected natural prawn culture environments by making a study of the food items of the prawns as well as the availability of these items in the particular environments. In this connection it may be stated here that although the selectivity of food of certain fishes have been studied, the selectivity of food in prawn in relation to the quantity of food organisms present in the habitat has not been attempted by previous workers.

### *Material and methods*

In the study conducted the stomach contents of three species of penaeid prawns collected from different prawn culture ponds during a period of 3 months from May to July 1983 were analysed to find out their food preference. Altogether 385 specimens of *Penaeus indicus*, 187 of *Metapenaeus monoceros* and 156 *Metapenaeus dobsoni* were studied. For the analysis of stomach contents the points method which is essentially a volumetric method was used. The samples came from 4 different ponds representing different environ-

ments located in different areas namely (a) Central pond of Prawn Culture Laboratory, Narakkal representing a perennial pond in which prawn stocking is done by wild specimens mainly by letting in tide water through sluice gate, (b) Valappu pond of the Lab to Land programme of CMFRI, where selective stocking of prawns and *Chanos chanos* is carried out, (c) Pasupakkar pond, a perennial pond of 3 ha area with single sluice to regulate entry of tide water and prawns stocked by this process and (d) Kannupillikettu, a large perennial pond of 40 ha area with 2 or 3 sluice gates for water regulation and stocking of prawns from the wild. From all these 4 stations prawns as well as mud samples were taken simultaneously along with water samples for hydrological studies. The mud samples were collected by using a core sampler made out of perspex plastic as a modification of Hope's corer. The core samples were preserved in 5% formaldehyde stained with Rose Bengal and macrobenthos sieved through 0.5 mm mesh were identified in order to study the food availability for the prawns in the different ponds.

#### Results and conclusion

Hydrographic parameters such as salinity, dissolved oxygen, temperature and pH of the sampling stations were also noted during the period of study. During May and June salinity and temperature showed higher values while in July, the monsoon month, their values declined. Dissolved oxygen value slightly increased in July and pH remained more or less constant.

The main food items of the prawns in the benthic fauna of these stations consisted of polychaetes, crustaceans like amphipods and decapods, annelids, molluscs and nematodes. Benthic food items identified in the mud samples were Polychaetes (*Prionospio polybranchiata*, *Heteromastides bifidus*, *Paraheteromastus tenuis* and *Neridae*), amphipods (*Quadrivisia bengalensis*, *Grandidierella bonneiri*), decapods, other annelids, molluscs, and nematodes. Molluscs were represented by bivalves (*Pandora flexuosa*) and gastropods (*Littorina* sp.) and decapods by mysids. Difference in the benthic food item, both quantitative and qualitative were observed in the different ponds and in different months.

Seasonal variations in quantity and quality as well as pond to pond variations were observed during the period of investigation. During premonsoon months where salinity is high, polychaetes were dominant. Amphipods, molluscs, nematodes, decapods and annelids came next in decreasing order of abundance. However, coinciding with salinity decrease and molluscs came into dominance.

A decrease in the abundance of crustaceans and polychaetes and simultaneous increase in other items like molluscs is observed in the low saline month of July. Although seasonal changes in the food organisms like crustaceans and polychaetes in the different environment were reflected in the gut contents of the prawns, it was not seen to have a definite correlation.

Significant variations were found from pond to pond especially when salinity decreased. Marked pond to pond difference was also observed in the abundance of the different organisms. The quantity of animals represented in the samples was maximum in the pond of the Prawn Culture Laboratory, Narakkal, Valappu pond, Pasupakkar pond and Kannupillikettu were next in the order of abundance. The species representation also was slightly different in the different ponds.

In *Penaeus indicus* crustaceans formed the major constituent in the stomach (21.11%). Detritus (19.64%) vegetable matter (18.41%) and polychaetes (16.49%) were the next important food items. *Penaeus indicus* is omnivorous with a preference for animal matter especially Crustacea. Seasonal variations do occur in the food items of the species. During premonsoon, crustaceans formed the major constituent of the food while during monsoon period detritus was preferred. Mollusca also increased in the monsoon season coinciding with the availability of this item in increased quantities during the period. Pond to pond variation was not found to be very significant, suggesting that *P. indicus* takes the same type of food and probably selects these items in their environment. The acceptability of food items has a correlation with the food item in the environment.

In the case of *Metapenaeus dobsoni*, detritus (43.22%) constituted the major food component with vegetable matter (24.12%) coming next. Thus, the species is essentially a detritus feeder. Seasonal variation was not very much noticeable as in the other species, with the changes in the food organisms in the environment. This is probably due to the fact that detritus is the major food item of the species. Area wise variations between stomach contents of specimens caught from different ponds were also not very much noticed.

In *Metapenaeus monoceros* vegetable matter including filamentous algae and angiosperm tissues (38.32%) contributed to the important food item. Crustaceans (23.47%) was next in abundance. Polychaetes constituted about 11.09%. This species, therefore,



feeds mainly on vegetable matter with second preference to smaller crustaceans. When the crustacean remain show a noticeable decrease in the stomach contents during monsoon month the percentage was made up by algae, vegetable matter and detritus. Disappearance of diatoms from the gut contents was observed during late July.

Statistical analysis for food selectivity in the case of *Penaeus indicus* and *Metapenaeus monoceros* were attempted, but no evidence was found to suggest that the species are selective as regard to the choice of the food organisms.

Among the three species of prawns studied it is clearly evident from the study that there may not be any likelihood of competition for food. Eventhough the gut contents showed dominance of certain food organisms, it cannot be taken as a token of definite selectivity and it may be concluded that availability of food organisms in the environment determines the selectivity of prawns.

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# 12

## ENDOCRINE CONTROL ON OSMOREGULATION IN THE PRAWN *PENAEUS INDICUS* H. MILNE EDWARDS

### *Introduction*

The regulation of cell volume is a crucial aspect in the conquest of biotopes and establishment of organisms in the aquatic environment with fluctuating osmolarities. The crustaceans on account of their variety of habitats at each stage of their life cycle have to regulate the concentration and ionic composition of body fluids in relation to the outside medium.

### *Objectives*

Osmoregulation in penaeid prawns have been studied by different workers. The influence of endocrines on ionic regulation in prawns is an area with very few investigations. Hence this work was carried out to understand the origin of the endocrine factor and their control/role on osmoregulation in the prawn *Penaeus indicus*.

### *Material and methods*

Though not an exhaustive study, the experiments were based on the eyestalk ablation technique since it is supposed to be the seat of hormones in crustaceans. By removing the eyestalk it is assumed that the factor controlling the levels of ions in the body fluid of the animal is being removed.

Experiments were designed to investigate the role of the eyestalk factors in the regulation of the ions, viz. Sodium, Potassium and Chloride. The effect of eyestalk ablation on Ammonia excretion was also studied.

The test parameters were studied using experimental and control animals. Four batches of twenty *Penaeus indicus* were maintained and in the first batch one eyestalk was removed. Simultaneously in the second batch bilateral ablation was performed. In the third and fourth batch also both eyes were removed, but injected with eyestalk extract and distilled water respectively to find out the origin of the endocrine factor controlling osmoregulation.

### Results

The haemolymph sodium level increased in *Penaeus indicus* as a result of the removal of eyestalks (from 422 mEq Na/L to 447 mEq Na/L). The increase was more pronounced in the case of bilateral ablation (55 mEq Na/L). Injection of eyestalk extract into destalked animals helped them restore the level almost similar to that of normal animals.

Eyestalk ablation resulted in an increase in the haemolymph chloride level also. An increase of 43 mEq Cl/L and 54 mEq Cl/L were observed under unilateral and bilateral ablation respectively. When the eyestalk extract was injected into bilaterally ablated animals the chloride level decreased and attained normal values (314 mEq Cl/L).

The behaviour of potassium ion was also similar to that of sodium and chloride.

Unilateral and bilateral removal of eyestalks induced an increase in ammonia excretion. In the control unablated animals the ammonia excreted was  $96.1 \pm 1.9$   $\mu\text{g/g}$  prawn, whereas for the bilaterally ablated animals it was  $135.77 \pm 5.0$   $\mu\text{g/g}$  prawn. In the destalked animal with eyestalk extract injection there was a reduction in the total level ( $104.07 \pm 3.74$   $\mu\text{g/g}$  prawn) in twentyfour hours reaching almost normal values.

### Conclusion

The regulatory patterns exhibited by the experimental animals indicate that the eyestalk contains factors affecting ionic balance. An ion decreasing factor may be present in the eyestalk which on removal causes an upsurge from normal ionic values. The effect of hormone extract in bringing down the increasing ionic level provide additional testimony to this fact.

Ammonia excretion by *P. indicus* increased when both eye stalks were removed. The eyestalk ablation effectively isolates the neuroendocrine system which may be elaborating the factors controlling ammonia excretion. The enhanced excretion rates may probably be due to the degradation of aminoacids to meet the enhanced metabolism induced by the treatment.

Thus, we find that endocrine factors—eyestalk principles—have a control on ion regulation in *Penaeus indicus*.

*Papers communicated for publication based on this dissertation*

KIRON, V. AND A. D. DIWAN. The influence of eyestalk ablation on the haemolymph sodium concentration in the prawn *Penaeus indicus* H. Milne Edwards. *Indian J. Fish.*

—AND ——. Chloride ion regulation in the prawn *Penaeus indicus* H. Milne Edwards after eyestalk ablation. *Ibid.*

—AND ——. The influence of eyestalk factors on Ammonia excretion in prawn *Penaeus indicus* H. Milne Edwards. *Comp. Biochem. and Physiol.*

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## STUDIES ON THE DIURNAL VARIATION OF CERTAIN ENVIRONMENTAL PARAMETERS IN CULTURE POND

### *Introduction*

Water quality management forms an integral part of aquaculture, at times this can also pose challenges. It is not farfetched to say that nearly all problems that arise in aquacultural systems are the result of degradation of water quality. In this context knowledge of water chemistry is vital. The aquatic environment is a dynamic one in which various physico-chemical factors vary with seasons and time, and the variations are caused invariably the result of interactions between them. It is common knowledge that diurnal variations in important parameters like temperature, oxygen and pH are critical in providing an ideal ecosystem to the cultivable organism. Such informations gathered will ultimately help in the successful water quality management leading to profitable harvest. The present study was taken up under this concept.

### *Material and methods*

This study was conducted during April to August, 1983 from brackishwater culture ecosystems at Narakkal near Cochin in the southwest coast of India. Samples were collected from two ponds and a feeder canal of the Narakkal Prawn Culture Laboratory of CMFRI which is separated from Arabian Sea by about 280 m of land strip and is connected by a canal to the Cochin Backwater. Water samples from surface were collected directly while the bottom samples were collected by a bottom water sampler at about 10 cm above the substratum.

## Results

The diurnal variation of atmospheric temperature was irregular, whereas in the surface and bottom water the trend was regular throughout the period. The atmospheric temperature was minimum (29°C) at 0400 hrs and maximum (31.4°C) at 1200 hrs. The surface water temperature was minimum at 0800 hrs (33.1°C) and maximum (34.8°C) at 1400 hrs. There was a general trend that always the surface water temperature was higher than atmospheric and bottom water temperature. But between 0200-0600 hrs the bottom water temperature was higher than that of surface. In the pre-monsoon period the atmospheric temperature was maximum (31.4°C) at noon while surface water maximum (34.8°C) was at 1400 hrs and the respective minimum were 29.0°C and 32.4°C at 0400 hrs and 0600 hrs respectively. A sudden change of 2.0°C at around 0800-1000 hrs and at 0200-0400 hrs in surface water was noticed. During the monsoon period the maximum temperature showed no significant change, but the water was warmer at 1600 hrs. The minimum temperature showed a change. The minimum for surface and bottom were 30.9°C at 0400 hrs and 30.7°C at 0800 hrs respectively. The difference between surface and bottom temperature were about 1.0-1.5°C while that of atmosphere and the surface was 3.2-5.0°C.

The pH ranged from 8.2 to 9.4 during the period of study. During the pre-monsoon the surface water recorded a maximum of 8.66 pH at 1600 hrs and minimum of 8.30 pH at 0600 hrs, while that of the bottom varied from 8.20 to 8.62 pH. During the monsoon the water was slightly alkaline. The pH of the surface water ranged from 9.24 at 1600 hrs to 8.80 at 0600 hrs, while that of the bottom water was 9.11 at 1400 hrs to 8.69 at 0600 hrs.

During the pre-monsoon period the Eh showed a minimum of -72 mV at 0600 hrs with a range of -24 mV. The bottom water had a maximum of -74 mV at 1400 hrs and minimum (-66 mV) at 0600 hrs. In the monsoon period, surface water had a maximum Eh of -132 mV at 1800 hrs. The bottom water readings fluctuated between -127 to -98 mV during 1600 and 0600 hrs.

Tides were semidiurnal. The difference between high and low water were 56, 95, 97, 82, 91, 83 and 70 cms respectively on the days of observations during April to August period.

The minimum oxygen level of 1.65 to 2.19 ml/l found in the early morning between 0400-0800 hrs kept on increasing till 1600-

1800 hrs and reached a maximum of 6.94 ml/l at 1700 hrs. A sudden increase of 2-3 ml/l during 0800-1000 hrs was noted, while a sudden decline of 3 ml/l was also observed during 2200-0400 hrs. The bottom water had about 0.4 ml/l more oxygen at 0600 hrs than that of surface during the premonsoon period, while the difference was less marked during the monsoon season.

The diurnal variation in salinity and nutrients were irregular. The nitrite concentration in the surface water varied between 1.06 and 2.92  $\mu\text{g at/l}$  while that of the bottom from 1.14 to 3.96  $\mu\text{g at/l}$ . The nitrate in surface water varied from 1.96 to 52.00  $\mu\text{g at/l}$ . In the bottom it varied between 1.76 and 56.00  $\mu\text{g at/l}$ . In the pre-monsoon period the phosphate in the surface and in the bottom fluctuated between 7.32 and 30.55  $\mu\text{g at/l}$  and 3.01-25.33  $\mu\text{g at/l}$  while during the monsoon it varied from 0.74 to 15.55  $\mu\text{g at/l}$  and 0.52 to 57.00  $\mu\text{g at/l}$ . The surface water phosphate concentration exceeded that of bottom water on 27th June and 23rd July. In the monsoon time the surface concentration exceeded the phosphate concentration of the bottom water in the late evening hours of April and June.

Ammonia concentration too was found to fluctuate highly and irregularly from nil to 195.00  $\mu\text{g at/l}$ . The concentration varied even in between the ponds at different periods as well as on specific dates of study.

The statistical analysis showed that the temperature variations at two hourly interval were not marked in both periods at different layers. Diurnal oxygen changes were marked (1.60-3.29 ml/l) during 1000-1200 hrs of both the seasonal periods. pH values had the maximum change of 0.20-0.24 during 1000-1200 hrs during pre-monsoon seasons whereas the variations at two hour interval were negligible during monsoon. In the case of Eh the variations were -5 to -14 during 1000-1200 hrs in pre-monsoon. At 0400 hrs the changes in all the above mentioned parameters were high and marked.

The correlation between the surface and bottom layers for temperature, pH, Eh and dissolved oxygen were highly significant ( $P \geq 0.01$ ) and positive. The correlation coefficient were 0.86, 0.99, 0.99 and 0.95 respectively. Relationship among atmospheric and surface temperatures were highly significant (0.85). Oxygen values depended on the surface temperature of water in both the premonsoon and monsoon periods and were positive (0.560, 0.396).

The correlation between dissolved oxygen, pH and Eh of surface and bottom layers were significant at 5% level. There is an unusual phenomenon noticed in the case of tidal amplitude vs. salinity during the sampling days of different ponds. In most of the cases the salinity did not depend on tidal amplitude. The relationship of tidal amplitude and salinity were positive and as well as negative in the same day at different ponds and not significant.

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## STUDIES ON SOILS OF SOME BRACKISHWATER PRAWN CULTURE FIELDS AROUND COCHIN

### *Introduction*

In recent years great emphasis has been placed on the scientific management of mariculture of fishes and shellfishes. From earlier studies it is well known that the benthic organisms form food for the fishes and prawns cultured in brackishwater ponds. Thus benthic productivity becomes an important prerequisite for obtaining high yield. The benthic productivity is in turn dependent on the nature and fertility of the bottom soil. It is observed that from the same area different ponds show differences in productivity and in the same pond productivity changes from season to season.

### *Objectives*

Keeping the above facts in mind and that there is no investigation on the soil profile and their physicochemical nature in culture ponds of the Cochin region, an attempt was made to study the important parameters of soil which have important bearing on the productivity of a pond.

### *Material and methods*

For the study three different ecosystems such as seasonal field, perennial field and brackishwater canals in coconut grooves where prawns and fishes were cultured, were selected. The seasonal fields chosen were the four ponds with a central canal with average depth

ranging from 0.5 to 1 m at Valappu. Soil samples were collected once a month from January to June 1982 at fifteen selected stations. The perennial fields (with an average depth of 0.5 to 1 m) chosen were the demonstration ponds at Prawn Culture Laboratory (PCL) of the Central Marine Fisheries Research Institute at Narakkal. Both soil and water samples were collected from the western pond and pond B. Only soil samples were collected from pond C, inner and outer canals. Samples were collected once a month in March and April and twice from May to June, 1982. Other than the above ponds, soil samples were collected from two coconut grooves and one seasonal prawn field (pokkali) from April to June 1982. The coconut grooves had a water depth of 0.5 m to 0.75 m while that of pokkali field was about 0.3 m. The soil samples were collected by using a van Veen grab (0.05 m<sup>2</sup>) and bottom water samples were collected with the help of a bottom sampler. The soil samples were collected in wide mouth polythene bottles and closed tightly. Water samples were analysed for temperature, pH, dissolved oxygen and salinity. Soil organic carbon was estimated by Walkley and Black's rapid titration method and soil calcium, sodium and potassium by flame photometer. Soil Eh and grain size analyses were also carried out on representative samples.

#### *Results and discussion*

The temperature of bottom waters in the fields of Valappu increased from a minimum of 27.5°C in January to reach a peak of 31.2°C in May. This trend was reflected in other ecosystems. The salinity of the bottom water in the perennial ponds of Valappu recorded a progressive increase from 20.2 in January to 31.1 ppt in March which represented the peak, and then showed a steady decline to record 2.5 ppt in June. In other ecosystems the maximum salinity was observed in April. The pH was generally found to be between 7 and 8 during January-March in all ecosystems and thereafter showed a gradual increase to reach the maximum value in May and June (8.3 to 9.3). This increase might be due to the effect of monsoon rain. The oxygen values in all ecosystem was from 2.7 to 8.6 ml/l. Generally the dissolved oxygen was on the lower side in Valappu, while Narakkal PCL ponds and coconut grooves showed consistently higher values throughout the sampling period.

The sediment organic carbon values did not show any definite seasonal trend during the period of study. In general, coconut

grooves showed minimum values and maximum values were recorded from pokkali fields (6.5%). This could be due to regular addition of organic matter in the form of paddy stalks left over from paddy cultivation.

The statistically significant negative correlation between bottom dissolved oxygen and organic matter at Valappu and Narakkal PCL ponds brings to light the vital role of oxygen in the degradation of organic matter. The correlation between the three exchangeable actions estimated and organic carbon in all the three ecosystems with only one exception of potassium of pokkali fields indicates the dependance of these nutrient cycles on the carbon cycle as also reported by Worbel [1967, *FAO Fish Rep.*, 44 (3): 153-163].

Wide variations were observed in all the ecosystems with respect to monthly average values of soil sodium (6875 to 35,000 ppm), Calcium (2425 to 12,050 ppm) and potassium (198 to 1141 ppm). Generally, the values were higher at Valappu. Significant positive correlations were found between salinity and sodium, and salinity and calcium indicating the important role of salinity in determining the amounts of exchangeable cations present in brackishwater pond soils.

A decline in redox potential from April to May was the general trend observed in all the ecosystems and the range of monthly values observed was from -14 to -278. The Eh showed positive correlation with organic carbon and this is in agreement with the findings of earlier workers. On the basis of grain size the soils at Valappu, Narakkal PCL ponds, coconut grooves and pokkali fields could be classified as silty clayey loam, loamy sand, sandy and sandy clay respectively. Greater fertility of the soil at Valappu and pokkali fields as against the soil of other fields is attributed to the predominance of clay and silt in the soil composition. In core samples from Valappu, organic carbon and exchangeable cations were higher in the surface than in the subsurface fraction. In Narakkal PCL ponds such a stratification was not evident.

#### *Conclusion*

In conclusion it may be said that among the different prawn culture fields studied, pokkali fields ranks first in the fertility status of the soil in respect of all the soil parameters monitored except soil

texture. The fields of Valappu comes second, followed by Narakkal PCL ponds and coconut grooves.

*Paper communicated for publication based on this dissertation*

ESWARA PRASAD, P. AND V. K. PILLAI. Studies on soils of some brackishwater prawn culture fields around Cochin. *CMFRI Bulletin*, 28 (2) (in press).

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## STUDIES ON BENTHOS IN PRAWN CULTURE FIELDS OF VYPEEN ISLAND, COCHIN, KERALA

### *Introduction*

Several studies are available on benthic organisms and their distribution, abundance, species diversity and seasonal variation in the inshore waters, estuaries and backwaters of India. However, only limited studies have been made on the abundance and distribution of benthic organisms in the brackishwater fish/prawn culture fields. In the context rapidly developing coastal aquaculture in the country and considering the importance of benthos in sustaining the productivity of the culture ponds, the present investigation was taken up to study the macrofaunal constituents of benthos in certain prawn culture fields of Vypeen Island near Cochin during January-June, 1982 along with important hydrological and sedimentological parameters which influence them.

### *Area of study*

The study was carried out in three different ecosystems prevalent at Narakkal (Cochin), namely, two earthen ponds in the field complex belonging to the Prawn Culture Laboratory of the Central Marine Fisheries Research Institute, representing the perennial culture system; canals in the coconut grove representing typical brackish water environment in a coastal plantation and an earthen field (Pokkali field) where paddy and prawns are cultured seasonally following the traditional method. All these ecosystems were situated at a distance of 300 m from sea.

### *Material and methods*

Mud samples for macrobenthos were collected fortnightly from fixed stations using a 'van veen' grab with a volume of 0.05 m<sup>3</sup>.

The animals were separated from the mud by sieving with a 0.5 mm round mesh sieve and preserved in 5% formalin with rose bengal to provide a colour contrast between the benthos and sediment fractions. Wet weight of macrobenthos was taken in the laboratory. Dry weight was estimated after washing the animals in distilled water to remove extraneous salts and drying at 60°C for 16 hours. The number of animals was estimated as per 0.1 m<sup>2</sup> and the wet and dry weights per 1 m<sup>2</sup>.

The water temperature was recorded by an ordinary thermometer with 0-50°C graduation. The bottom water was collected by a bottom water sampler. Salinity was estimated by Mohr titration and dissolved oxygen by winkler method (Strickland and Parsons, 1968, *Bull. Fish. Res. Bd. Canada*, 167). The nitrite-nitrogen (NO<sub>2</sub>-N) was determined by spectrophotometric method (Kennethbendschneider and Robinson, 1952, *J. Mar. Res.*, 11 (1): 87-91), while the nitrate nitrogen (NO<sub>3</sub>-N) by the method described by Mullin and Riley (1955, *Anal. Chem. Acta.*, 12: 464-480).

The turbidity of the water was measured using a Jackson turbidometer and pH of the bottom water by an 'Elico' pH meter.

Sediment samples collected by the grab were analysed for grain size following the combined sieving and pipette method (Krumbein and Pattijohn, 1938, *Manual of sediment petrography*, pp. 549); Eh and pH using 'Elico' pH meter with separate electrodes and organic carbon by Walkley and Black's (1934, *Soil Sci.*, 37: 29) rapid titration method with slight modification. After adding 10 ml of potassium dichromate and 20 ml of sulphuric acid, the samples were digested in a constant temperature water bath at 100°C for fifteen minutes instead of keeping on asbestos sheet for 30 minutes.

The percentage of organic carbon in the method was calculated as follows:

$$\frac{V_1 - V_2}{W} \times 0.003 \times 100$$

Where V<sub>1</sub> = Volume of potassium dichromate; V<sub>2</sub> = Volume of Ferrous sulphate and W = weight of the soil sample. Every ml of potassium dichromate accounts for 0.003 g of carbon.

#### *Results and discussion*

*Temperature:* The temperature of the bottom water in the perennial fields, canals in the coconut grove and in the 'pokkali'

field was varying between 28°C and 33°C, 27°C and 33°C and 29.2° and 32°C respectively. Lower bottom temperature was observed in June in all the ecosystems.

*Salinity:* Salinity of the waters in all the ecosystems showed an upward trend from January to April (11 to 30‰), but it declined to reach the lowest value of 5‰ in the perennial pond and canal water. Maximum salinity value was recorded in April.

*Dissolved oxygen:* The dissolved oxygen values of the bottom water showed fluctuation from 3 ml/l to 7 ml/l in different ecosystems. In the perennial field, peak dissolved oxygen value was recorded in June and the minimum (3 ml/l) in January. In the coconut grove canals the dissolved oxygen was seen fluctuating between 4 ml/l and 6 ml/l. In the 'pokkali' field, however, it was relatively higher (5 ml/l – 7 ml/l).

*Nitrate:* The nitrate of the water in the perennial field and the coconut grove canals was seen increasing gradually from January to reach a peak (2000–3000 µg/l) in June. In the 'pokkali' field, relatively high values were recorded throughout the period as compared to the other ecosystems.

*Nitrite:* In the perennial field, nitrite values of the water remained generally low upto April (0.2 µg/l to 40 µg/l). Peak values were recorded in June (842 µg/l to 976 µg/l). In the canals in the coconut groves the nitrite content was higher than in the perennial fields. In the pokkali field it was not stable, but also higher than that in the other ecosystems.

*Turbidity:* The turbidity of the water column was low in all the ecosystems upto the end of April and thereafter showed a progressive increase reaching a peak in June (200 Jtu) with the onset of the southwest monsoon.

*pH:* The pH of the water showed fluctuation between 6.8 and 8.8, the maximum value being recorded in June. The pH of the mud in the perennial field was near neutral during the study period, while in the coconut grove canals it was ranging from 6 to 7. In the 'pokkali' field, the pH was found to be acidic during January–April, the range being 5.9 to 6.3.

*Organic carbon:* The percentage of organic carbon was found to vary between 0.03 to 9.6% in different ecosystems. While it was

consistently high in one of the perennial fields and coconut groove canals, it was low in the other fields. In the 'pokkali' field, organic carbon was 9.6% in May.

**Redox Potential (Eh):** The redox potential in different ecosystem was found between -30 and -350. While the distribution of Eh was comparable in the perennial and seasonal fields, it was relatively higher in the coconut groove canals.

**Grain size:** The grain size analysis of the mud samples showed three textural classes. One of the perennial ponds and canals in the coconut groove showed muddy texture while in the other it was sandy. In the pokkali field, the texture of the mud was clayey.

#### *Bottom fauna*

In the perennial ponds, polychaetes *Dendronereis aestuarina* and *Notophygos* sp. represented the major macrofaunal composition. In the coconut groove canals and 'pokkali' fields, polychaetes were not encountered throughout the period of observation.

Nematodes were present in large numbers in all the ecosystems, April being the period of peak abundance in the perennial ponds and canals in the coconut groove.

Tanaidaecids were represented by two species viz., *Apseudes chilkaensis* and *A. gymnophobium*; and they were found abundantly in perennial ponds during February-April. In the coconut groove canals their numbers were few, while they were completely absent in the 'pokkali' field.

Amphipods were recorded throughout the period in perennial ponds and coconut groove canals. They were represented by *Melita* sp. In the 'pokkali' field, amphipods were absent.

Bivalves were represented mainly by *Pandora flexuosa* in all the ecosystems. Gastropods constituted by *Littorina* sp. were abundant in May, in the perennial pond. However, in the coconut groove canals and 'pokkali' field, the gastropod population did not show much variation.

The total population count was relatively high in May in perennial ponds, in April in coconut groves and in March in the 'pokkali' field. The total population of macrobenthos decreased in all the ecosystems by June with the onset of southwest monsoon.



Generally the benthic biomass showed a progressive increase from January, attaining the peak in April and then a decline in June in all the ecosystems.

The statistical analysis to find out the correlation between the various environmental parameters and the benthic biomass revealed that the temperature, pH of the water, pH of the mud and nitrate did not show significant correlation with the biomass at 5% level. Whereas salinity and the organic carbon content had a positive correlation at 1% level of significance. The redox potential and nitrite, however, showed negative correlation with biomass at 5% level of significance.

From the multiple regression analysis assessed by the standard partial regression coefficient, the main factors which limit the abundance and distribution of benthic macrofauna were found to be salinity, followed by organic carbon, redox-potential and nitrite nitrogen in the perennial fields and in one of the canals in the coconut grove. In the other canal, redox-potential acted as the main limiting factor. In the 'pokkali' field, however, nitrite was the principal limiting factor followed by organic carbon, salinity and redox-potential.

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## ECOLOGY OF MEIOBENTHOS IN SELECTED CULTURE FIELDS AROUND COCHIN

### *Objectives*

In recent years, renewed interest to understand the dynamic nature of meiobenthos and their relationship with the co-existing or overlying benthos and the associated economically important demersal organisms was aroused, particularly in the context of more and more effort put in to explore the economically important groups such as prawns through culture means in suitable ecosystems. It is now known that the food of juvenile and adult prawns consists predominantly of benthic organisms. While certain information is available on the distribution and availability of macrobenthos in the culture fields, there is very little information on the meiobenthic community, although it forms an important constituent in the benthic food chain. In few of this, and to understand their role in the dynamic ecosystem where prawns and other brackishwater fishes are cultivated, the present investigation was taken up.

### *Material and methods*

The investigation was carried out at Narakkal (76° 14'E; 10° 03'N) about 10 km northwest of Cochin. Three different ecosystems were selected for the present study viz., a 'pokkali' field, a canal in the coconut grove and one of the experimental ponds of the Prawn Culture Laboratory of the Central Marine Fisheries Research Institute.

Core samples of the sediment were taken by a modified Hope corer. The device consists of a 40 cm plexiglass tube having an

inner cross sectional area of 10 cm<sup>2</sup>. Lines for measuring the length of the core samples were inscribed at 2 cm intervals. The upper open end of the tube could be closed with a rubber stopper. A small circular air intake hole of 1 cm diameter is drilled through the side in the upper half and covered with a rubber sleeve. Sediment samples were taken by pushing the lower end of the core into the sediment, closing the upper end with the stopper and then withdrawing the samples. Sample is taken by holding the corer vertically and rolling down the rubber sleeve over the air intake hole. In order to study the meiobenthic population, samples of the sediment from the top two cm and the underlying two cm were taken separately, preserved in 4% formaldehyde and stained by 'Rose bengal' stain. Samples were also collected for estimating the nutrient content in the meiofaunal layer. Bottom water retained above the sediment layer held in the corer was taken for the analysis of dissolved oxygen content by winkler's method. Salinity estimates were made by collecting water from the same depth. pH and Eh of the bottom water was measured using a 'Century CP 901' digital pH meter. Turbidity was measured using a Jackson turbidometer.

Sediment analysis was carried out by using sieves of A.S.T.M. Nos. 18, 35, 60, 120 and 230. A known weight of the dried sediment was wet sieved initially through a 62 $\mu$  sieve to separate the silt and clay from sand fraction. Subsamples of the silt and clay fraction were taken and analysed according to the method of Milne (1971) as follows:

$$P = \frac{100}{W_1} \left( \frac{1000 W_2}{V} \right)$$

where P=% wt. of clay; W<sub>1</sub>=total weight of sediment; W<sub>2</sub>=weight of the dried fraction of the clay and V=volume of the subsample. From the data obtained thus, the mean diameter of the sediment sample was calculated by finding the graphic mean (Mz) as:

$$Mz = \frac{(\phi 16 + \phi 50 + \phi 84)}{3}$$

where  $\phi 16$ ,  $\phi 50$ ,  $\phi 84$  are the 'phi' equivalent at each indicated cumulative percentage. The spread of the distribution of the 'sorting' was calculated probability graph method for inclusive standard deviation  $\sigma - I$  as:

$$\sigma - I = \frac{(\phi 84 - \phi 16)}{6.6} + \frac{(\phi 95 - \phi 5)}{6.6}$$

where  $\phi$ -I=inclusive S.D., and  $\phi$  84,  $\phi$  16,  $\phi$  95 and  $\phi$  5 the 'phi' equivalents of the corresponding percentages.

Dissolved oxygen and salinity content of the samples where determined by the method suggested by Strickland and Parson(1968). Carbon content of the sediment, taken as a measure of organic matter was estimated by chromic acid method and the percentage carbon in the sediment was calculated as:

$$\% \text{ carbon} = \frac{3.951}{0.150} \left( 1 - \frac{T}{S} \right)$$

where 'T' in the volume of  $(\text{NH}_4)_2 \text{Fe} (\text{SO}_4)_2$  used up for sample and 'S' volume of the same used up for blank titration.

Available phosphorus was extracted by 0.03 N  $\text{NH}_4\text{F}$  and estimated by the colorimetric reading of the blue colour developed by chlorostannous reduced molybdophosphate at 650 nm.

Chlorophyll 'a' content of the sediment was determined by extracting chlorophyll 'a' from the subsample with 90% Acetone with the addition of 1 ml  $\text{Mg CO}_3$  suspension for 20 Hrs in darkness, and the extinction values were read spectrophotometrically; the concentration of chlorophyll 'a' was determined using the formula:

$$11.6 E_{665 \text{ nm}} - 1.31 E_{654 \text{ nm}} - 0.14 E_{630 \text{ nm}}$$

where E=extinction coefficient at indicated wavelengths after correction at 750 nm.

Meiobenthic biota were separated by sieving the sediment through 1 mm and 62  $\mu$  sieves and faunistic composition studied.

Correlation coefficients ('r') between the meiobenthic population density and each observed parameter were calculated in order to assess the influence of environmental parameters on the distribution and abundance of the fauna.

#### Data

The range, mean and S.D. of different parameters in the three ecosystems studied during February to August, 1983 are as follows:

ECOSYSTEM 1. *Canals in the coconut grove*

Parameter	Range	Mean	Standard deviation
Temperature (°C)	27.0–31.5	28.74	1.31
pH	6.97–8.18	7.85	0.32
Eh (MeV)	–67 – –06	–48.93	19.44
Salinity (ppt)	2.94–36.36	22.32	11.10
Oxygen (ml/l)	1.74–4.33	2.61	0.79
Organic Carbon (gm %)	0.6733–4.4258 (0.8318–5.268)	1.79 (2.0354)	1.30 (1.24)
Available phosphorus (µg/gm)	3.9006–6.8572 (2.3333–6.4287)	5.4244 (4.4504)	1.30 (1.08)
Chlorophyll 'a' (µg/gm)	0.0151–0.1949 (0.0706–0.3016)	0.1296 (0.1505)	0.07 (0.06)
Population of meiofauna (nos/10 cm <sup>2</sup> )	2 – 348 (0–54)	136 (22.3)	91.2 (14.6)

Figures in parenthesis indicate values for the 2–4 cm layer of the sediment.

ECOSYSTEM 2. *Pokali field*

Parameter	Range	Mean	Standard deviation
Temperature (°C)	28.0–30.5	29.5	0.95
pH	7.9 – 8.27	8.09	0.13
Eh (MeV)	–52 – –73	–63.5	6.97
Salinity (ppt)	17.84–30.50	25.88	4.45
Oxygen (ml/l)	0.1424– 3.23	1.92	1.05
Organic carbon (gm %)	0.8318–1.4591 (0.7726–5.106)	1.0929 (3.51)	0.21 (1.65)
Available phosphorus (µg/gm)	3.2381–9.0476 (4.1900–5.9048)	6.2953 (4.86)	2.16 (0.64)
Chlorophyll 'a' (µg/gm)	0.0906–0.4341 (0.0576–0.4794)	0.2382 (0.2913)	0.13 (0.15)
Population of meiofauna (nos/10 cm <sup>2</sup> )	24–97 (0–8)	56 (5)	37.49 (3.07)

### ECOSYSTEM 3. *Experimental pond*

Parameter	Range	Mean	Standard deviation
Temperature (C°)	28.0-33.5	30.36	1.60
pH	7.79-8.77	8.08	0.33
Eh (MeV)	-47 -- 103	-62.4	19.03
Salinity (ppt)	4.31-36.36	23.09	9.58
Oxygen (ml/l)	2.02-4.69	3.25	0.76
Organic carbon (gm %)	0.7528-2.7654 (0.4357-1.688)	1.4140 (1.0981)	0.59 (0.48)
Available phosphorus ( $\mu$ g/gm)	3.5238-6.8266 (3.9504-5.7143)	4.7780 (4.6805)	1.02 (0.69)
Chlorophyll 'a' ( $\mu$ g/gm)	0.0210-0.2310 (0.0277-0.1118)	0.1165 (0.0679)	0.052 (0.026)
Population of meiofauna (nos/10 cm <sup>2</sup> )	6-226 (4-48)	128 (24)	75.5 (13.67)

Figures in parenthesis indicate values for the 2-4 cm layer of the sediment.

Correlation co-efficients obtained between each environmental parameter and the meiofaunal population density in the culture systems.

	Canals in coconut grove	Experimental pond
Salinity	0.5269	0.2881
Oxygen	0.4699	0.6664
Available phosphorus	0.4485	0.4103
pH	0.4214	0.1333
Carbon	-0.3472	-0.0514
Temperature	0.3310	0.3086
Chlorophyll 'a'	0.3137	0.0175
Eh	-0.1716	0.1283

### *Results and conclusions*

The variation in the temperature was 27.0°C to 33.5°C and high values were recorded during summer months. The temperature of the bottom water was relatively high in the experimental pond as compared to that in the other two ecosystems. pH values in all the three ecosystems ranged from 6.97 to 8.77 indicating more alkaline nature of the environment. Eh values varied from -47 to +06 in these ecosystems. Salinity showed marked variations and

it ranged from 2.94‰ to 36.6‰ in the coconut grove, 4.31-36.36‰ in the experimental pond and 17.84 to 30.5‰ in the 'pokkali' field. Drastic decrease in the salinity coincided with the onset of monsoon in June. Oxygen concentration in the bottom water was relatively less in the 'pokkali' field (0.14 to 3.23 ml/l), when compared to that in the other two ecosystems, where it varied from 1.74 to 4.33 ml/l in the water of the canals in the coconut grove and 2.02 to 4.69 ml/l in the experimental pond. Turbidity in all the three ecosystems was generally below 100 J.T.V. indicating that the amount of suspended matter was low in these waters.

The sediment in the 'pokkali' field was found to be clayey in nature, while that of the experimental pond and canals in the coconut grove were sandy silt. Organic carbon in the top 2 cm layer evinced much fluctuation in the sediment in the canals in the coconut grove (0.67 to 4.43 g%) where as it was low in the other two ecosystems (0.75 to 2.76). In the bottom 2 cm layer the carbon content showed relatively high values in the sediment in the canal of the coconut grove (0.83 to 5.27 g%) and experimental pond (0.77 to 5.11 g%) whereas in the 'Pokkali' field, it was found to be low (0.44 to 1.69g%). Available phosphorus in the top 2 cm and bottom 2 cm layers of these ecosystem did not evince much difference. However, the phosphorus concentration in the upper layer ranged from 3.90 to 9.04 µg/gm and its values in the bottom 2 cm layer varies from 2.33 to 6.43 µg/gm. Chlorophyll 'a' content in the 'pokkali' field was relatively higher (0.05 to 0.43 µg/gm) whereas in the canals in the coconut grove, the chlorophyll 'a' values ranged from 0.02 to 0.25 µg/gm and in the experimental pond the values varied from 0.02 to 0.23 µg/gm. Relatively high values of chlorophyll 'a' content of the sediment was observed during April and May in these ecosystems.

Benthic meiofauna comprised mainly of nematodes, polychaetes, harpacticoid copepods, lamellibranch spats, cumaceans and foraminiferans. Among the different groups of animals encountered, nematodes formed the major component, forming 82.5% of the meiofauna in the 'pokkali' field, 72.0% in the canals of the coconut grove and 60.0% in the experimental pond, followed by lamellibranch spats in the experimental pond (24.2%) and in the coconut grove (6.1%) and harpacticoid copepods (13.5%) in the 'pokkali' field.

A drastic decrease in population density was observed with the onset of monsoon and the concomitant reduction in salinity. The number of benthic meiofauna showed differences in the three ecosystems with much variation.

Vertical distribution of the meiofauna in the 0 to 2 cm layer (upper) and 2 to 4 cm layer (lower) of the sediment in the three ecosystems was studied. Majority of the meiofaunal constituents was found to occupy the upper 2 cm layer of the sediment in all three ecosystems. 87% of the population in the 'pokkali' field, 84.4% in the canals of the coconut grove and 79.5% of the total meiofauna in the experimental pond were recorded from this layer.

The results of statistical analysis showed that salinity followed by dissolved oxygen influence the abundance and distribution of meiofauna in the canals of the coconut grove. In the experimental pond, available phosphorus followed by temperature acts as the controlling factors. It was also noted that the available phosphorus and temperature of the medium might influence the meiofauna independent of other environmental parameters studied.

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**COMPARATIVE KARYOLOGICAL STUDIES ON  
PRAWNS *PENAEUS INDICUS* H. MILNE EDWARDS AND  
*PENAEUS MONODON* FABRICIUS**

*Introduction*

Understanding of the basic genetic make-up of the animal is a very important pre-requisite in aquaculture karyological studies of the candidate species can indicate the chromosomal compatibility of the parents and predict the success of hybridization. Rapid genetic gains can be attained through chromosomal engineering techniques like polyploidy and gynogenesis. The sub-lethal effects of pollutants, mutagens and radiation can be assessed from chromosomal damages. For all the above studies knowledge of basic karyotype is essential. Studies on prawn karyotyping have been limited since crustacean chromosomes are more in number and small in size.

*Objectives*

The present study was carried out (1) to standardise the methodology with particular emphasis on penaeid prawns and (2) to determine the diploid chromosome numbers of *Penaeus indicus*, *P. monodon*, *Metapenaeus monoceros* and *M. dobsoni*.

*Material and methods*

Juveniles of these species in the size range 50-90 mm were used for the experiments. Two types of methods are commonly used for karyotype studies, viz., squash and air-dried methods. The

basic steps in both the above methods are, a mitotic inhibitor is administered intra-muscularly or into the media. After allowing sufficient time for the action of the mitotic inhibitor, the animal is dissected and the chosen tissue is subject to hypotonic treatment to swell the tissues. To facilitate swelling, the tissue is fragmented. Then the cells are allowed to burst and are stained.

For standardisation the parameters chosen were the mode of fragmentation of the tissue, hydrolysis with hydrochloric acid, choice of hypotonic media, duration of hypotonic treatment, choice of fixative, duration of fixation and mode of bursting of cells. By following the standardised squash method, hundred metaphase spreads were counted for each of the *Penaeus* spp. and fifty for the *Metapenaeus* spp. From these counts, the mode was established and the diploid chromosome number determined.

#### *Results and discussion*

From the different combination of sequential treatments attempted for the standardisation of the air-dried method, the best combination yielding the maximum metaphase spreads were selected. Of the tissues tested gills were found superior to testis and hepatopancreas. When the gills were chopped instead of homogenisation, more number of intact individual cells could be recovered. Of the hypotonic media tested, 0.7% KCl was found better. The tested hypotonic duration varied from 3 hrs to 10 minutes and a minimum duration of 10 minutes was found to result in adequate swelling without bursting. A overnight fixation in 3:1 methanol acetic acid at -2°C was found superior to shorter duration of fixation or other fixatives. When the slurry of fixed cells were put gently into the slide and airdried, more spreads were obtained than with heat drying or ignition of the cells.

In a similar manner the best combination of sequential treatments for the squash method were selected. Hypotonic treatment for 15 minutes duration of the entire gills followed by fixation in 3:1 ethanol: acetic acid was found ideal. Hydrolysing the fixed tissue in 1 N HCl for 6 min. at 60°C followed by rinsing in distilled water was found to yield better spreads. After this, storing the tissue in 45% acetic acid for 24 hours at 1°C followed by chopping and squashing in glacial acetic acid resulted in more spreads than in other combination of treatments attempted.

From the present study it is seen that the bursting of the cells is determined by (i) the hypotonic treatment and (ii) the final steps

of the treatment *i.e.*, squirting, dropping or putting the cells gentle on to the slides and (iii) method of drying *i.e.* heat drying, flamy drying or air-drying. The relationship between the hypotonic treatment and these final steps was found to be a crucial one. A slight variation in either of these causes prematurely burst cell resulting in scattered chromosomes and incomplete metaphase plates, or unburst cells in which chromosomes are not clear. Though the air-dried method could be standardised to a certain extent, the complete metaphase plates were not obtained. Complete metaphase spreads were obtained by squashing, but details could not be studied as experienced by Farmer (1974, *Crustaceana*, 27 (1): 17-20) in *Nephrops norvegicus*.

#### Results and discussion

In this study the results indicate that the squash method provides more recoverable figures while air-dried method though more risky, would provide better quality spreads. It would be best to make karyological studies on prawns using both methods simultaneously. The air-dried method would be more useful for more detailed study and the chromosome counts could be confirmed by the squash method.

From the chromosome counts it was found that the diploid number of *Penaeus indicus*, *P. monodon* and *Metapenaeus monoceros* were 66, 84 and 92 respectively. The modal diploid number for *M. dobsoni* was found to be between 82 and 86.

From the literature available the chromosomal count of decapod crustaceans was found to lie between 48 to 246. It is evident that the high number of chromosomes observed in the present study is comparable to the diploid chromosome number of *P. aztecus*, *P. duorarum*, *P. setiferus* and *P. japonicus* are 88, 88, 90 and 92 respectively from earlier studies. The diploid number of 84 in *P. monodon* more or less comes close to the range of 88 to 92 observed in other species of the genus *Penaeus*. But the diploid number of *P. indicus* being only 66 falls very much short of the chromosomal number of other *Penaeus* spp. The significance of this can only be determined by further studies on other species of the genus *Penaeus*.

*P. indicus* and *P. monodon* are candidate species for hybridisation trials. But the present study has revealed that there is a great disparity in chromosomal number between both the species. Therefore the chances of a fertile hybrid between these species is less.

The results of the present study have to be confirmed using more number of animals and by counting a greater number of metaphase spreads. More study in this direction would be useful in hybridisation and other genetics studies on the Indian penaeid prawns.

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## PRAWN SEED TRANSPORTATION

### *Introduction*

In any large scale culture operation one of the foremost requirements is the availability of seed as and when required by the farmer. Whether the seed is produced in the hatchery or collected from the wild, they are to be transported and distributed in the farm site where they are further cultured. This necessitates transportation of seed to shorter or longer duration depending on the distance of the farm site from the site of seed production or collection centre. Any large scale mortality of seed during transportation thus incurs heavy economic loss to the enterprise.

### *Objectives*

In India much priority is laid over the culture of prawn and its propagation in low-lying areas along the coast. However, the success of propagation and intensive prawn culture would largely depend on a viable seed transportation technique. The exploitation of brackishwater prawn seeds from the nature and the modern techniques of transport are yet to be perfected in the country. So far only very little is known about the requirements for the transportation of cultivable brackishwater prawn in India, as also the possible causes of mortality in transit. The present study was therefore undertaken on the transport of *Penaeus Indicus* seeds under oxygen packing in plastic bags with a view to elucidate the important environmental and physiological factors that contribute to mortality in a closed system and to define the optimum density of prawn seeds which could be transported in a unit volume of water.

### Material and methods

For the present investigation on transportation, postlarvae of *P. indicus* were obtained from the Prawn Culture Laboratory of the Central Marine Fisheries Research Institute at Narakkal, Cochin. The experiments were carried out using eight days old (PL 8, 8-12 mm) sixteen days old (PL 16, 13-15 mm\*) and twenty days old (PL 20, 14-18 mm\*) postlarvae. Clear sediment free brackishwater of salinity 22-25 ppt, which was filtered using a biological filter and stored in plastic pools and kept well aerated was used for acclimatisation of seeds, as well as for all the transportation experiments. The transportation bag used in the present study was of 4 litre capacity (19.4 cm diameter, 16.5 cm height) made of H/gauage, soft, non toxic PVC with a firm base and a double safety internal valve to stop oxygen from leaking out even if the top opens.

Seeds were collected from the nursery pools using velon screen nets. They were kept in 35 litre capacity plastic basins containing brackishwater. Animals were fed with prepared feed and kept in these basins for 2 to 3 hours for acclimatisation. Desired number of seeds were collected from these basins, individually counted and transferred to the buckets containing brackishwater. Before packing, the bags were filled with one litre of the brackishwater. The seeds from the buckets were then collected in a soft cloth kept immersed in water in a basin. The excess food materials and excretory wastes were removed. The seeds alone were then quickly transferred to the bags. For this a wide mouth funnel was used, the tip of which was kept immersed in the water in the transportation bag. The seeds collected together in the centre of soft cloth, was gently kept inverted over the mouth of the funnel and washed down with one litre of the brackishwater. Thus, volume of the water was made upto two litres in the transportation bag. Once the seeds have been transferred to the bags, pure oxygen was bubbled through the water for one minute. The air above the water column was expelled by squeezing the top portion of the bag, and the bag was tightly closed. The space above the water was now filled with oxygen through the valve. In all the experiments the quantity of oxygen filled in the bags was kept constant, being two litres.

Three different sets of experiments, each in triplicate, were conducted using PL 20, PL 8 and PL 16. For the first set of experiments PL 20 was used. Seven different densities such as 25, 50, 100, 150, 200, 250 and 300 postlarvae per litre (PL/l) were used.

\*Length measured from the tip of rostrum to the end of the telson.

Experiments were carried out for three different periods of 24 hrs, 36 hrs and 48 hrs respectively. For the second set of experiments eight densities of 50, 100, 150, 200, 250, 300, 350 and 500 PL/1 were used for three different durations of 24 hrs, 36 hrs and 48 hrs. Brackishwater having a salinity range of 22-25 ppt was used for the first two sets of experiments. Third set of experiments was carried out using sea water of 33 ppt salinity. Experiments were carried out in seven densities for a period of 48 hours. Dissolved oxygen,

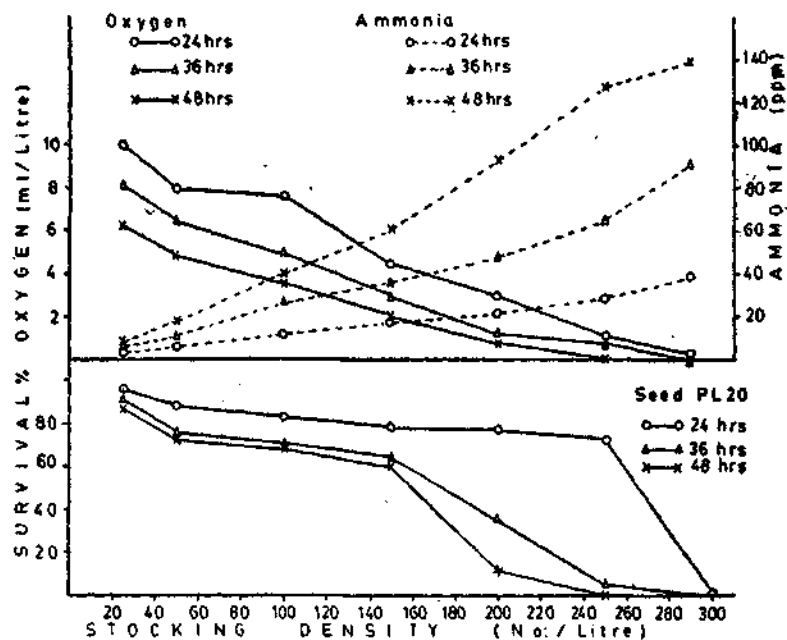


Fig. 1. Survival rate of *Penaeus indicus* seeds of 14-18 mm size at various stocking densities for different durations and the final oxygen and total ammonia concentrations after the close of the experiments.

pH and total ammonia of the water used for transportation were determined before the commencement of the experiment. After the duration of each experiment, the bags were opened and the surviving seeds counted. The water was analysed for dissolved oxygen, pH and total ammonia. Although the temperature was not controlled during the experiment, initial and final temperature of the water was noted.

## Results

In the first set of experiments with PL 20, in the 24 hours duration transportation with 25 PL/l the percentage survival was 96.6. With the increasing stocking density the survival rate was found to decrease gradually upto a stocking density of 250 PL/l. When the stocking density was between 150 and 250 PL/l the survival rate was above 70%. When the stocking density exceeded beyond 250 PL/l, the survival rate was found to decline suddenly with almost total mortality of the seed (Fig. 1). In the transportation of seed for 36 hours highest survival rate (92.6%) as expected was found in the lowest stocking density, but it decreased to 76.3% when the seeds were at a density of 50 PL/l. The mortality of seed between 50 and 150 PL/l was found to be gradual unlike the heavy mortality noticed when the stocking density was changed from 25 to 50 PL/l. Above 150 PL/l stocking density the survival rate decreased considerably. In the experiment of transportation of seed for 48 hours duration in different stocking densities the survival rate showed a similar pattern as in the case of the previous experiment for 36 hours duration. The survival rate of above 60% was obtained at 150 PL/l. In this series of experiments the initial temperature of the medium was at 30°C, pH 8.0, oxygen 15.46 ml/litre and total ammonia 0.15 ppm.

In the second set of experiments with PL 8, the survival of seed during 24 hours transportation was above 80% in the stocking densities between 50 and 150 PL/l. It was about 73% when 250 PL/l stocking density was used for transportation. As the stocking density increased to 300, 350 and 400 PL/l the survival rate declined to about 69%, 62% and 57% respectively. In the experiment on the transportation of seed for 36 hours duration above 80% survival was recorded only when the stocking density was 50 PL/l. It was varying between 70–77% when the stocking density was raised to 150 PL/l and about 63–64% in the stocking density of 200–250 PL/l and above this the survival declined to less than 60%. In the case of 48 hours transportation, about 70% survival of the seeds was seen with a stocking density of 50 PL/l. In higher stocking densities appreciable mortality was recorded as compared to those in the other two experiments, the survival rate being between 50 and 60% in 100–200 PL/l, between 40–50% in the 250–400 PL/l (Fig. 2). In the beginning of the experiment the temperature of the medium was 30°C, oxygen 16.01 ml/litre, pH 8.0 and total ammonia 0.15 ppm respectively.

In the third set of experiments with PL 16, observations were made only for 48 hours duration transport. Results showed that



93.3% survival was noticed when the seeds were transported at the rate of 25 PL/1. Ten per cent decrease in survival was noticed when the stocking density was doubled. About 72% survival of the seeds was recorded at 150 PL/1. Above this stocking density the mortality was appreciable. Salinity of the medium was 33 ppt, initial temperature 27°C, pH 8.02, Oxygen 15.12 ml/litre and total ammonia 0.10 ppm.

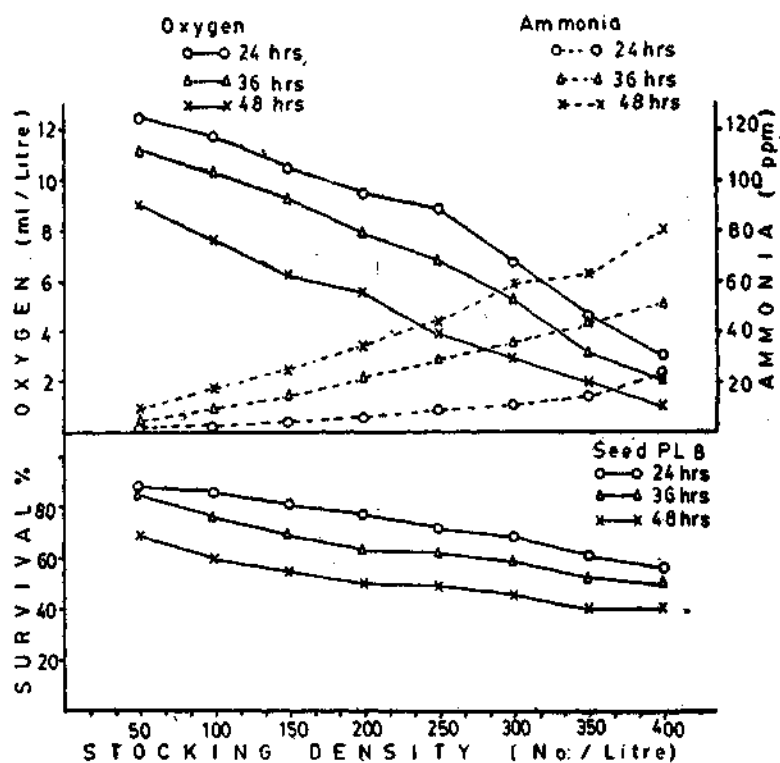


Fig. 2. Survival rate of *Penaeus indicus* seeds of 8-12 mm size at various stocking densities for different durations and the final oxygen and total ammonia concentrations after the close of the experiments.

In the present study the temperature was not controlled and the entire transport of seed was carried in the ambient temperature (27-30°C). The temperature of the medium at the beginning of the experiment was 30°C in the first two experiments conducted with

brackishwater medium while it was slightly lower (27°C) in the transportation carried out in sea water medium. At the end of the experiment the temperature registered an increase of 1°C in all the experiments. In the third set of experiments with PL 16 seeds the survival was comparatively better than the first set of experiment with PL 20. One of the reasons for this better survival might have been the lower temperature during the course of transit.

It is known that when the prawn seeds are transported under oxygen pressure dissolved oxygen is not found to be the main cause of mortality. It is also known that seeds of *P. indicus* can survive in healthy conditions when the oxygen level is at 2.5 ml/litre although the lethal level is at 0.2 ml/litre or below. In the first set of experiment depletion of oxygen was observed only in the higher stocking densities of over 200 PL/l. This is particularly evident in the longer duration experiments for 36 hours and 48 hours. Thus, during 24 hours of transportation, when the stocking density was 300 PL/l oxygen level came to 0.37 ml/litre and the survival was 1.6% (Fig. 1). It is evident that in the longer duration transportation (36 and 48 hours) for the same stocking rate almost complete mortality would have happened at 24 hours due to the effect of high levels of CO<sub>2</sub> (indicated by low pH) and ammonia, afterwards the dead and decomposing animals alone would have contributed to oxygen depletion. In the second and third set of experiments the oxygen level was above 1.3 ml/litre even in higher stocking density when the seeds were transported for longer duration of 48 hours (Fig. 2).

In the experiments carried out with brackishwater the pH fell below 7.0 at 70% survival level in the first set of experiments. Below 6.6 level considerable mortality was recorded in the first set of experiment and in the second set survival was below 45%. The decrease on pH noticed in higher stocking densities in all the observations in the present experiments may be due to the accumulation of CO<sub>2</sub> bringing in marked decline in pH. During the present set of experiments, most of the seeds died when the total ammonia reached above 80 ppm.

### Conclusions

The following conclusions were drawn from the present set of experiments.

For PL 20 (14–18 mm) a total of 250 seeds per litre could be transported for 24 hours with 70% survival; for longer duration this survival rate was obtainable at 100 seeds per litre. For smaller

seeds (8-12 mm) the stocking density could be increased by 50 per litre for 24 and 36 hours duration (i.e. 300 and 150 seed per litre respectively). But for 48 hours duration, survival was poor even in lower stocking densities.

When the experiments were compared, it was observed that the survival rate of small size seeds was relatively low, especially for longer duration transport. This has been attributed to cannibalism. When PL 16 was transported in sea water 70% survival was obtained for a period of 48 hours when the stocking density was 150 seed per litre. This better survival when compared to that of experiments conducted with PL 20 might have been due to the reduced temperature during the course of the experiment when PL 16 was tried.

With the ratio of oxygen maintained at 1:1 as in the present investigation, depletion of oxygen was not found to be the main cause of mortality.

Mortality of seeds has been related to reduced pH (due to accumulation of  $\text{CO}_2$ ) and increase in ammonia considerable mortality was recorded when pH fell below 6.6 and when ammonia level exceeded 70-80 ppm.

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## SERUM CHOLESTEROL, PROTEIN AND GLUCOSE CONTENT IN *ETROPLUS SURATENSIS* (BLOCH)

### *Introduction*

With increasing interest in aquaculture especially finfish culture, the study of fish tissue chemistry becomes most important. There is a need to identify the biochemical components, their normal levels and their fluctuations due to variation in environmental parameters. This facilitates the identification of the optimum environmental and nutritional conditions that can maintain the culture species in the healthiest condition possible. The major hazard in intensive culture systems is the breakout of diseases. The first response of the fish to any pathological attack is the deviation of the levels of most of the biochemical levels from the normal conditions. This change is mainly manifested in the blood. Here comes the importance of the study of blood chemistry. Fish blood chemistry is thus found to be an important tool of the fishery biologists and serves as an indicator of the physiological condition of the fish. In this aspect, the analyses of blood alone is not enough, since blood is only the transporting medium of the various biochemical components. Therefore the analysis of other tissues such as the muscle, liver, etc. becomes necessary to observe the original variations of the biochemical parameters.

### *Objectives*

The present study has been conducted in *Etroplus suratensis* with the following objectives.

1. To establish the normal ranges of the selected biochemical parameters such as protein, glucose, free aminoacids, lipid and cholesterol in serum; moisture, protein, glucose, glucogen, free aminoacids and lipids in the muscle and liver.

2. To determine the effect of starvation on these biochemical parameters.

3. To determine the interrelationship between these parameters and that between environmental and biochemical parameters.

#### *Material and methods*

The seasonal changes in the biochemical parameters were estimated in the brackishwater adult fishes *E. suratensis*. Monthly collections of fishes were made during the period from March to August 1983 from a brackishwater canal at Valappu, near Narakkal in Vypeen Island. Immediately after capture by cast net, the fishes were transferred to canal water in plastic tubs and given aeration for two hours. Five individuals of more or less equal length were selected. Blood was drawn by cardiac puncture into heparinized syringes. Serum was separated by centrifugation at 3000 rev/min. for 10 min. Samples of liver and muscle were drawn from the same fish. At each sampling, salinity and oxygen content were determined. Starvation experiment was carried out in two batches lasting for 10 and 15 days. For the initial values a control sample of five fishes were dissected and serum, liver and muscle samples were drawn. At the end of the experimental period five healthy individuals were sampled. The estimated biochemical parameters were protein, glucose, lipids, free aminoacids and cholesterol.

#### *Results*

The monthly variations in all the estimated serum biochemical parameters were found to be not statistically significant. Therefore the ranges of observed values is given and for protein it was 2.02 to 3.4 gm/100 ml, for glucose 50.4 to 96.2 mg/100 ml, free amino acid 0.93 to 1.88 gm/100 ml, lipid 0.01 to 0.08% and for cholesterol 245-382 mg/100 ml. Among these parameters, the maximum variation between months and also within a month was seen in the case of serum lipid and the coefficient of variation ranged from 4.7 to 45.1%.

In the muscle only lipid showed statistically significant differences between months. A peak mean value of 1.54% was observed in

March and lowest level of 1.1% in July. Of the other parameters, moisture ranged from 72 to 78.5%, protein 12.8 to 18 mg/100 mg, glucose 1.89 to 4 mg/100 mg, glycogen 0.025 to 0.08 mg/100 mg and free amino acid 0.32 to 1.4 mg/100 mg.

In the liver only glycogen and free amino acid did not exhibit statistically significant differences between months and these ranged from 0.148 to 1.98 mg/100 mg and 0.82 to 1.8 mg/100 mg respectively. The moisture content exhibited a peak value of 75.5% (March) and a minimum value of 64.6% (June). A peak protein value of 13.42 mg/100 mg was observed in April and the lowest level of 10.66 mg/100 mg was observed in June. A peak value of 4.8 mg/100 mg (May) and a minimum value of 3.8 mg/100 mg (July) was observed in the case of glucose. In the case of liver lipid a high level of 3.46% was observed in March and a lowest level of 2% was observed in July.

### *Conclusion*

Statistically significant decrease in all the estimated biochemical parameters in serum, muscle and liver was observed for both periods of starvation. Moisture content showed a significant increase. With a view to understand the mobilisation pattern of protein, fat and glycogen from the different organs and their breakdown into glucose and free amino acid for utilisation, inter correlation between the estimated parameters within a tissue and between tissues was calculated. Significant interrelationships were observed between most of the parameters in the serum, liver and muscle in the starvation experiment. But in the monthly analyses, the individual constituents of the muscle did not show any significant inter-relationships. In the liver, a positive correlation was observed between the moisture content and lipid content ( $r=0.497$ ,  $P<0.05$ ). Both in monthly samples and starvation experiment, between tissues there was positive correlation between muscle glycogen and serum glucose, muscle glucose and serum glucose, and liver protein and muscle protein. A positive interrelationship between muscle protein and serum protein was observed only in the monthly samples. Positive correlations between liver glycogen and muscle glycogen, liver glycogen and muscle glucose and muscle and serum free amino acid was observed only in starvation experiments.

A comparison with the range of biochemical values reported in literature for other fish indicates that the present values except for muscle glycogen lie within this range. Muscle glycogen values are comparatively lower. Seasonal values in serum and muscle

with the exception of lipid showed no significant variation unlike in other fishes. The reasons could be the short period for which the study was carried out or the fact that *E. suratensis* does not undergo starvation during any particular season. Though salinity showed decreasing values throughout the study with minimum values in August, none of the biochemical parameters including free aminoacid in the three tissues showed any parallel changes. Increase in free aminoacid in fishes has been reported with increase in salinity. In the present study muscle lipid, liver moisture, protein, glucose and lipid values are at a minimum during June and July. Salinity as the major factor responsible for this change can be ruled out since these parameters increase when the salinity shows the minimum value in August. The only possible reason is that it is due to the second spawning that occurs in monsoon season (Jaya-prakash, 1980, *Seafood Export Journal*, 12 (11): 1-4). Starvation for short duration of ten days results in significant decrease in all biochemical parameters. A comparison of the monthly values with the starvation results indicate that they do not lie within the same range, indicating in nature *E. suratensis* did not undergo starvation during the period under study. The positive correlations between the biochemical parameters within a tissue and between tissues indicate that the depletion is uniform in all the tissues and not at a particular tissue. Finally, the present study has brought out the importance of the study of biochemical parameters of the liver in assessing the physiological state of fishes.

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## STUDIES ON THE PITUITARY GLAND OF SELECTED CULTURABLE FINFISHES

### *Objectives*

In the present study an attempt has been made to investigate the morphological details of the pituitary glands of three economically important mullets namely *Valamugil cunnesius* (Valenciennes, 1836) (Thunberg, 1792), *Liza parsia* (Hamilton, 1822), *Mugil cephalus* (Linnaeus, 1758) and the catfish *Tachysurus maculatus* (Thunberg, 1792). The different cell types of the gland were located and identified by using the latest staining techniques.

### *Material and methods*

The live mullets were collected from the Chinese dip nets at the Cochin barmouth and the catfish in fresh and chilled condition from the Cochin Fisheries Harbour. All the specimens studied were immature in either the 1st or 2nd stage of maturity. Dissections were made in the laboratory on anaesthetised and chilled fishes under dissection microscope to take out whole pituitary gland located in the depression at the base of the skull. *In situ* drawing of the gland of each species was made to illustrate the gross morphology.

For preparation of tissue sections, the gland along with a portion of the brain was fixed in aqueous Bouin's fixative and Helly's fluid for periods ranging from 24-28 hours. After fixation, the tissues were washed thoroughly in 30% alcohol and dehydration was done by transferring the tissues to the higher grades of alcohol



in sequence and finally subjected to cold impregnation with xylene (saturated with wax) for about 30 minutes. Embedding and blocking procedures were done using paraffin wax of melting point 58-60°C. Transverse sections of the whole pituitary glands were taken at 6-8  $\mu$  thickness.

Microphotographs showing the different topographical regions and the cell types of the pituitary were taken with the Olympus photomicrographic system - Model PM 10 AD using ORWO NP 27 400 ASA black and white film. Colour photographs were taken using Kodacolor 11 colour negative film of 100 ASA speed.

For the identification of the different cell types the following staining procedures were adopted:

- i. Periodic Acid Schiff (PAS-Orange G with celestine blue haemalum sequence,
- ii. Chromium-haematoxylin-phloxine
- iii. Mallory Heidenhain's
- iv. Orange-Fuchsin-Green
- v. Camoron and Steele method

In the chromium-haematoxylin-phloxine method, the sections were kept in haematoxylin solution for 45 minutes until the beta cells became deep blue. In the PAS-Orange G procedure with celestine blue haemalum sequence, instead of Meyer's haematoxylin, Cole's haematoxylin was used. While following the PAS procedure, rinsing the sections three times in 0.5% aqueous sodium metabisulphite was not done, but instead a thirty minutes washing under the running tap was given. For Mallory Heidenhain's method the slides were stained in azocarmine solution at 52°C in oven for two hours. In the O.F.G. Method of Slidders, a 0.2% solution of light green was used instead of 1.5% light green.

#### *Morphology of the gland*

*Valamugil cunnesius* : The pituitary gland is a compact and slightly cone shaped structure located in the sella turcica, a concavity of the sphenoid bone. The gland is situated ventral to the brain, immediately behind the optic chiasma and above the capillary network, the *rete mirabile*. The gland is encapsulated by the dura

mater. It is attached to the floor of the infundibulum by a very short stalk and hence the pituitary gland is of the leptobasic type. Since the stalk enters the gland from the anterior side it can be described as cranio-leptobasic. The pituitary gland in this case, is dull white in colour and is surrounded by connective tissue and fat. The pituitary is vascularised by the branches of the internal carotid artery.

Microscopical observation of the whole pituitary section shows that it is composed of four well defined regions viz. the frontal lobe the *rostral pars distalis*, the middle lobe, the *proximal pars distalis* and the distal lobe the *pars intermedia* (together constituting the adenohypophysis) and the neurohypophysis above innervating more in *pars intermedia*.

*Liza parsia* : The basic arrangement of the pituitary gland is similar to that of *V. cunnesius*. The pituitary gland of *L. parsia* is a pinkish and cone-shaped structure situated ventral to the brain immediately behind the optic chiasma and in front of the capillary network, the *rete mirabile* situated in the middle region of the lobus inferiori. The gland is located in the sella turcica, a concavity of the sphenoid bone. A definite stalk is present for the gland and hence it is of the leptobasic types and since the stalk enters the gland from the anterior side it can also be described as cranio-leptobasic. The gland is surrounded by connective tissue and fat.

The pituitary gland, when examined closely under the microscope showed four definite regions: the rostral pars distalis, the proximal pars distalis, the pars intermedia and the neurohypophysis. The major portion of the gland is occupied by the pars distalis; neurohypophysis occupies a comparatively lesser area and the remaining portion is occupied by the pars intermedia. The neurohypophysis is seen in the sections as a triangular region composed of a meshwork of nerve fibres and contain comparatively few cells. This region is seen to be surrounded by the pars distalis and the pars intermedia.

*Mugil cephalus* : In *M. cephalus* the general pattern of the enlodgement of the pituitary gland is the same as in the above two fishes. The pituitary gland is much larger in size when compared to the above two fishes and is dull pink in colour, typically cone shaped and compact, with a small depression on the dorsal side. It is located in the sella turcica, the concavity of the sphenoid bone. It is situated on the ventral side of the brain in between the lobus inferior, below the optic chiasma and above the *rete mirabile*.

The gland is of the cranio-leptobasic type and is found to be surrounded by connective tissue and fat.

In the transverse sections, the pituitary gland appears to be heart-shaped and four distinct regions could be distinguished as described for earlier fish. The neurohypophysis is very narrow and the majority of the area is occupied by the pars distalis (proximal and rostral). The distal end of the pituitary gland is occupied by the pars intermedia.

*Tachysurus maculatus* : The pituitary gland is more evolved compared to the pituitary glands of three fishes described above. The deep pink, oval pituitary gland is enlodged in the concavity of the parasphenoid. It is situated just behind the optic chiasma in a notch between the two inferior lobes and above the *rete mirabile*. The gland is of the cranioleptobasic type, with a short stalk with which it is attached to the floor of the infundibulum. The pituitary gland and the parts of brain surrounding it is found to be covered by layers of fat and connective tissue. The gland lies in close approximation with the brain and its dorsally raised region fits into a notch in the brain formed between the two inferior lobes. The pituitary is bilaterally symmetrical with a slightly convex and smooth ventral surface.

Three different regions could be recognised in the transverse sections of the entire pituitary gland namely; a centrally located neurohypophysis, the pars intermedia surrounding and innervated by the nerve fibres of the neurohypophysis and the distally located pars distalis. Majority of the area is occupied by the pars intermedia and the rest by the neurohypophysis.

In the present study, the general pattern of the position of the pituitary gland is the same in the four species studied. The gland is a compact structure located in the sella turcica, a concavity of the sphenoid bone. It is situated ventral to the brain, immediately behind the optic chiasma and above the capillary network, the *rete mirabile*. The gland is encapsulated by the dura mater and attached to the floor of the infundibulum by a short stalk. Hence the pituitary gland is of the leptobasic type.

#### *Conclusion*

On the basis of the point of entry of the pituitary stalk into the brain, the leptobasic type is further divided into the dorsobasic,

craniobasic and caudobasic. In the four fishes of the present study, the cranioleptobasic condition could be observed.

The identification of the different cell types in the pituitary glands have been made possible by the characteristic arrangements and distribution of the different cell types. In teleosts the distribution of the cell types within the adenohypophysis is highly regular so that the topographical location of a cell type can support its identification on tinctorial grounds to a far greater extent than in the other vertebrate groups.

The histological details of the pituitary glands of the four fishes studied showed a common pattern.

The prolactin and TSH cells were observed in the rostral pars distalis and the ACTH cells in the interphase between the neurohypophysis and the rostral pars distalis. The STH cells and gonadotrops were observed in the proximal pars distalis and the MSH cells in the pars intermedia. The neurohypophysis was found to be composed of the neurosecretory nerve fibres arising in the preoptic nucleus and pituicytes were also observed.

*Papers communicated for publication based on this dissertation*

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## REPRODUCTIVE PHYSIOLOGY OF INDIAN SPECIES OF THE GENUS *PERNA* (FAMILY MYTILIDAE)

### *Objectives*

The reproductive physiology of the two species of Indian sea mussels, namely the brown mussel *Perna indica* and the green mussel *P. viridis*, has been investigated by a comprehensive approach to the problem. The major aspects of the study include ecophysiology of reproduction linking up the annual reproductive cycle of the animals with the ecological conditions of the natural mussel beds, biochemical and histochemical changes associated with reproduction, and the neurosecretory cycle in synchrony with the reproductive cycle. Some basic studies on gamete morphology and certain aspects of gamete physiology have been taken up. The experimental work deals with the influence of different feeding levels on gametogenesis and maturation, and the effect of ganglia ablation on spawning.

### *Materials*

The materials for the investigations on *Perna indica* were collected from the natural mussel beds at Vizhinjam, near Trivandrum and on *P. viridis* from Elathur, near Calicut. The period of observations extended from October 1981 to December 1982.

### *Observations and results*

Data on temperature, salinity, dissolved oxygen, turbidity, phytoplankton production and rainfall have been presented for

both the study areas. The annual range of the parameters for January-December 1982 period were: at Vizhinjam for brown mussel - temperature 24.3-29.3°C, salinity 32.55-36.68 ppt, dissolved oxygen 3.64-5.19 ml/l, turbidity 180-540 JTU and phytoplankton (Chlorophyll *a*) 2.15-25.57 mg/m<sup>3</sup>; at Elathur for green mussel - temperature 26.0-29.9°C, salinity 35.40-40.50 ppt, dissolved oxygen 4.16-5.94 ml/l, turbidity 100-940 JTU and phytoplankton (chlorophyll *a*) 3.31 to 22.0 mg/m<sup>3</sup>.

*Perna indica* was in reproductive phase during February to September and in the resting (vegetative) phase from October to January. Sex differentiation and gametogenesis commenced in February and active gametogenesis was noticed in March and April. Progressive maturation was noticed during May-July. While spawning in a small part of the population commenced as early as May, peak spawning took place in August. In September a vast majority of the population had entered the resting phase. The mean gonad index truly reflected the reproductive cycle and the highest index of 2.77 was obtained in July 1982 at peak maturation phase.

*Perna viridis* showed a primary reproductive cycle from July to November with the total adult population participating in the process and secondary cycle during January-March in which mussels of above 60 mm length alone participated. Each cycle was completed in a much shorter period than in *P. indica* with a greater degree of synchronisation in the population. Spawning took place from September to November with a peak in October in the primary reproductive cycle and was observed from January to March with a peak in March in the secondary cycle.

Relatively lower temperatures prevailed during the active reproductive season than during the non-reproductive season for both *P. indica* and *P. viridis*. In this respect the tropical species appear to be opposed to their temperate counterparts which spawn in higher temperatures. However, the secondary spawning of *P. viridis* is associated with higher temperatures, but the spawning is incomplete and partial.

Within the narrow annual variations of salinity in the open coastal waters, the reproductive phase of both *P. indica* and *P. viridis* was marked by lower salinity conditions and the non-reproductive phase by higher values. Individual processes of gametogenesis, maturation and spawning appeared to be triggered by progressive decrease in salinity.

A greater correlation was found between monthly rainfall and reproduction. In *P. indica* gametogenesis commenced in February with rainfall, but proceeded with precipitation. The heaviest rainfall in June (480.9 mm) took the process of maturation to be a peak and accelerated spawning. In *P. viridis* active gametogenesis in July–August coincided with 50% of the total annual rainfall during these two months and spawning commenced in early September with reduced rainfall. Rainfall, through its effect on lowering the temperature and salinity, appeared to influence the different processes of reproduction.

Reproduction also showed a high degree of correlation with availability of food. In *P. indica*, commencement of gametogenesis in February is preceded by a minor phytoplankton peak (chlorophyll *a* 11.02 mg/m<sup>3</sup>) and maturation is preceded by a major peak (chlorophyll *a* 25.57 mg/m<sup>3</sup>) in May. In *P. viridis* gametogenesis commenced in July with a subtle increase in phytoplankton biomass (chlorophyll *a* 5.99 mg/m<sup>3</sup>). Maturation was accelerated with increase in phytoplankton production and spawning commenced in September with a peak chlorophyll *a* value of 22.00 mg/m<sup>3</sup>. In this species, a minor secondary peak of chlorophyll *a* of 8.82 mg/m<sup>3</sup> in January appeared to support the secondary spawning. The larger animals (above 60 mm) which alone participated in this spawning might have been able to convert the food energy derived from the minor peak in January into reproductive effort as they have already attained a higher somatic growth than the smaller animals.

Localised pollution of coastal waters due to coconut husk retting near Elathur had an effect on the reproductive potential of *P. viridis*. Mussels at the site were all males without any exception and spawning was partial and incomplete. Pea-crab infestation in the indeterminate stage of *P. indica* resulted in non-development of reproductive tissue in the mantle. Infestation in the advanced stages of reproduction led to tissue damage at the site of 'lodging' affecting the reproductive potential of the mussel.

The structure of sperm and egg is similar in both the species. Under scanning Electron Microscope, the sperm of *P. indica* showed four distinct parts, namely acrosome, nucleus, middle piece and tail. The sperm, including the tail, measures 70  $\mu$ m. The salinity range 32.7–35.2 ppt appeared to be the most suitable for the survival of sperms of *P. indica*. The sperms were motile even after 3 h 30 min in the above range. The 50% sperm viability level was obtained approximately at 1h in 20.6 ppt, 1 h 45 min in 27.1 ppt, 2 h

30 min in 32.7 ppt, 2 h 15 min in 35.2 ppt and 1 h 45 min in 38.7 ppt. In *P. viridis*, 50% sperm viability level was recorded at 30 min, 1 h 15 min, 2 h 15 min, 2 h and 1 h in the above-mentioned salinities respectively. The 50% egg viability levels in the salinities 20.6, 27.1, 32.7, 35.2 and 38.7 ppt were reached respectively, approximately at 15 min, 2 h 30 min, 3 h 30 min, 3 h 30 min and 1 h 30 min and 1 h 30 min in the case of *P. indica* and 15 min, 4 h, 4 h 30 min, 8 h and 2 h 30 min in the case of *P. viridis*. Preliminary study on sperm preservation was carried out.

Biochemical studies on *P. indica* and *P. viridis* revealed that, the composition varied with the stages of maturity and also seasonally. The nutrient storage occurred in different tissues of the body. The protein was largely stored in the adductor muscle, carbohydrates as glycogen in the mantle and lipids in the digestive gland. The accumulation of these components was high during the nonreproductive season and also when food was in abundance. The excess energy, after providing for the maintenance metabolism, was stored and later utilised for reproduction. The protein level in the adductor muscle was comparatively higher than in the digestive gland and mantle. The lipid level was comparatively higher in the mantle of female than in the male, but glycogen level was higher in the latter. The lipid stored in the digestive gland was mobilised during active reproductive season especially during vitellogenesis. The glycogen stored in the mantle was used as the follicles developed in the mantle.

The gonad of the mussels was observed to be of glycolipoprotein in nature. The occurrence of acid mucopolysaccharides was confirmed. The lipid moiety in the gonad of mussel was of neutral lipid in nature. The male gonad contained more of glycogen than other carbohydrate components. The transfer of yolk precursors from different body tissues to the gonad was confirmed by histochemistry. The nutrients stored in the mantle connective tissue also was lysed and utilised during gametogenesis. Both adipogranula and vesicular connective tissue cells were observed in the mantle.

A 45-day experiment on *P. indica* to induce gametogenesis and maturation outside the natural reproductive season through feeding with mixed phytoplankton gave significant results. A good correspondence was observed among dry weight increase, digestive gland index and mean gonad index for different levels of ration (range 0.6-19.2  $\mu\text{g}$  chlorophyll *a*/animal/day). On a ration of 4.8-9.6  $\mu\text{g}$  chlorophyll *a*/animal/day, the mussels reached full maturity (mean gonad index 3) in 30 days. Statistical tests carried out on the data showed the relative significance of different feed levels in promoting



reproductive activity, somatic growth and digestive gland development. All the experimental animals giving positive response turned out to be males. It is inferred that oogenesis may require higher energy levels than those used in the treatments. The results of this experiment have practical value in controlled breeding of mussel for aquaculture purposes.

The neurosecretory cells (NSC) of a single type (pyriform) have been observed in the cerebral and visceral ganglia of *P. indica* and *P. viridis*. The pedal ganglia do not show presence of NSC. Four arbitrary stages of neurosecretory activity (NSA) have been described in the cerebral ganglia. Cytochemical study revealed that the neurosecretory material is acidic and glycolipoprotein in nature. The seasonal changes in the reproductive cycle synchronise with the changes in neurosecretory cycle. The sequences are similar in both the species although their annual cycle varies in respect to the months of the year.

Experimental evidence has been obtained on the role of neurosecretion in spawning of *P. indica* and *P. viridis* through extirpation of the ganglia of the central nervous system. The results were very similar for both the species. Bilateral cerebralectomy elicited greater spawning response than the unilateral ablation, approximately in the ratio of 4:1 and the females showed a slightly higher response than males. Visceralectomy too yielded spawning response, but on a much lower scale as compared to cerebralectomy. In the experiments, a certain amount of 'background' spawning, perhaps due to stress, occurred in the controls, but this response was very low. Pedalectomy elicited spawning response very close to the 'background' spawning in the controls. The spawning responses to various ganglion ablation treatments were statistically tested to understand their significance. Recovery process was poorer and slower in the viseralectomised animals than in the cerebralectomised ones, implicating the role of visceral ganglia in general metabolism. Mortality rates due to different treatments have been observed. The results of this study have provided further experimental evidence on the role of neurosecretion in the spawning of *Perna indica* and *P. viridis*.

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## CULTURE AND GROWTH KINETICS OF SELECTED NANOPLANKTERS

### *Objectives*

The development of mass cultures of phytoplankton as live-food, forms an integral part of hatchery systems in mariculture. Among such photosynthetic, microalgae, nanoplankters with cell dimensions of 50-60  $\mu$  and less are essential for providing the right type of food for early larval stages of bivalve molluscs. Hence the isolation and development of nanoplankters in axenic cultures *in vitro* conditions have been undertaken for further mass production.

### *Material and methods*

The present study on nanoplankton is based on the isolation and development of unialgal cultures from the inshore waters of Cochin, characterisation of their growth, assimilation products, eco-physiology and evaluation of nutritional quality. The work was carried out during the period 1980-1983. The nanoplankters were isolated and grown in the laboratory as batch cultures to study the increase in cell population, the photosynthetic pigments and physiological activity. The chemical composition of these organisms and their rate of excretion were also determined. The environmental factors - physical and chemical - that influence the growth of these cultures were defined by conducting independent experiments. Mass cultures of the isolated nanoplankters were raised indoor and fed to the larvae of edible oyster to test their suitability as live-food.

Taxonomic description of thirteen species of nanoplankters in the Cochin estuarine and coastal regions have been given. Of these, *Chromulina freiburgensis* Doflein and *Isochrysis galbana* Parke *forma nova* (referred as *I. galbana* (C.s.)) are new records for the Indian waters. The latter is considered as a new tropical form differing from the widely known temperate species.

The identification of *C. freiburgensis* and *I. galbana* (C.s.) was made from their morphological and anatomical features as obtained with light microscope and confirmed by Scanning Electron Microscope and analysis of photosynthetic pigments using thin-layer chromatography.

The various techniques and media employed in isolation and development of axenic cultures of nanoplankton and analysis of chlorophylls, chemical composition, rate of excretion as well as the methods employed to determine the role of various environmental factors on growth kinetics of these nanoplankters have been discussed.

The kinetics of growth of the present isolates *C. freiburgensis* and *I. galbana* (C.s.) were studied in detail along with two other nanoplankters *Synechocystis salina* and *Tetraselmis gracilis*. The growth of these species was defined by measuring the growth rate (as evidenced by the cell counts), the amount of chlorophyll and carotenoid pigments and photosynthetic production (given by the measurement of oxygen exchange and carbon-14 uptake) for a period of thirty days.

#### Observations

The various growth measurements of the above species showed that they exhibited peak growth and activity from two to six days of inoculation and then the growth rate declined gradually. Within 12 to 16 days all cultures attained stationary phase. As the cultures became one month old, their growth and activity reduced drastically with *I. galbana* (C.s.) and *S. salina* showing senescence while *C. freiburgensis* was more stable.

The amount of protein, carbohydrate and lipid of *C. freiburgensis*, *I. galbana* (C.s.), *S. salina* and *I. gracilis* was estimated at different phases of growth in culture. The relative proportion of the protein was high in all the species during the exponential phase of growth - being 58.4% of the dry weight in *C. freiburgensis* and

51.3% in *I. galbana* (C.s.). In *S. salina* and *I. gracilis* it was 41% and 54% respectively. The relative amount of protein decreased in the ageing cultures while that of carbohydrates increased. Lipids were comparatively less.

The amount of extracellular products released by the above nanoplankters during their phases of growth in culture was estimated by the carbon-14 method using a Liquid Scintillation Counter. It was observed that the rate of excretion increased with the age of cultures. During exponential phase, cultures of *C. freiburgensis* released 3% of the total carbon fixed to the medium while this fraction increased to 12.57% in stationary phase cultures. In the cultures of *I. galbana* (C.s.) there was an increase from 3.9% to 45.2% and in *S. salina* the range was from 38.91% to 64.5%. In *T. gracilis* the amount of excretion varied from 2.5% to 22.36% from the 5th to 16th day.

Salinity tolerance studies conducted with media of 14‰, 24‰ and 34‰ showed that the flagellates, *C. freiburgensis* and *I. galbana* (C.s.) grew best at 34‰, while *S. salina* showed better growth at 14‰.

The optimum pH for the species varied with *C. freiburgensis* and *I. galbana* (C.s.) growing best at a pH of 8 while *S. salina* proliferated better at a pH of 7 to 7.5.

The rate of carbon fixation by the nanoplankters was found to be affected by the density of culture. The flagellates had a lower optimal saturation density i.e.  $6-10 \times 10^5$  cells/ml compared to cultures of *Synechocystis salina* for which the rate of  $^{14}\text{C}$  uptake increased with cell concentration upto  $175 \times 10^5$  cells/ml after which there was decline. This difference could be related to the relative volume of the two species.

Cultures maintained on a light-dark cycle of 10:14 hours exhibited higher production rate when exposed to constant illumination. Similarly the light intensity supporting the maximum production of these cultures also did not differ significantly. The light adaptation of the species as defined by the constant  $I_K$  was found to be  $22-24 \times 10^{16}$  quanta  $\times \text{cm}^{-2} \times \text{sec}^{-1}$  for *C. freiburgensis*, *I. galbana* (C.s.) and *S. salina*.

The influence of temperature on the growth and activity of the nanoplankters was studied by growing them in thermostatically controlled water baths. The flagellates *C. freiburgensis* and *I.*

*galbana* (C.s.) exhibited maximum productivity at 30°C while *S. salina* was more active at 25°C. But the optimum range of temperature for growth was wider (20-35° C) for all the three species.

The thermal death point was 40°C for *C. freiburgensis* and *I. galbana* (C.s.) and 45°C for *S. salina*. *S. salina* survived longer periods (six months) of exposure to low temperature (ca 5°C) than the flagellates (3 months).

The rate of growth of *C. freiburgensis*, *I. galbana* (C.s.) and *S. salina* was studied with respect to varying concentrations of nitrate and phosphate in the culture medium and the nutrient requirement of these species were defined in terms of half-saturation constants for growth. Among the chrysomonads, *C. freiburgensis* was found to have a lower half-saturation constant and higher growth compared to *I. galbana* (C.s.) giving the former better chances for nitrate utilisation. *S. salina* was better equipped to compete with the flagellates at low nitrate levels.

The phosphate utilization capacity of *C. freiburgensis* was higher than that of *I. galbana* (C.s.), while *S. salina* was more or less at par with *C. freiburgensis*.

From the nutrient kinetic studies of the flagellates it was evident that *C. freiburgensis* has better chances of survival than *I. galbana* (C.s.) in culture.

Mass cultures of the present isolates *C. freiburgensis* and *I. galbana* (C.s.) were raised in 20 litre carboys. The flagellates grew and multiplied rapidly. In these mass cultures also *C. freiburgensis* showed more stability.

The acceptability of the two species *C. freiburgensis* Doflein and *I. galbana* (C.s.) to the molluscan larvae was tested. Newly hatched larvae of *Crassostrea madrasensis* fed separately with *C. freiburgensis* and *I. galbana* (C.s.) settled as spat in 17 and 19 days respectively. The results of the experiment showed that these flagellates were not only acceptable to the larvae, but also induced higher growth rate and larval survival compared to control feed i.e. *I. galbana* Parke, the temperate water strain that is widely in use in hatcheries. *C. freiburgensis* seems to be the more potent species for development as live-food in oyster hatcheries.

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\* Out of print.